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Proceedings of the Fifth Annual Aquatic Toxicity Workshop November 7-9, 1978 Hamilton, Ontario

Editors
P.T.S. Wong, P.V. Hodson, A.J. Niimi, V. Cairns,
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Fisheries and Marine Service

Technical Report No. 862

May, 1979

PROCEEDINGS OF THE FIFTH ANNUAL AQUATIC TOXICITY WORKSHOP

November 7-9, 1978, Hamilton, Ontario

Editors

P.T.S. Wong, P.V. Hodson, A.J. Niimi, V.W. Cairns and U. Borgmann

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This is the 20th Technical Report from the

Great Lakes Biolimnology Laboratory

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Workshop Chairmen - G. Craig, J. Klaverkamp, J.B. Wilson

Information Session - G. Craig, P.V. Hodson, R. Hoos, J. Klaverkamp, G. Leduc, T. Thacheray

ABSTRACT OF PROCEEDINGS

The proceedings of the Fifth Annual Aquatic Toxicity Workshop consists of 26 papers, 6 abstracts, a list of aquatic toxicity research programs in Canada and discussions from three workshop sessions.

Topics covered by the papers included environmental laws, waste treatment effects on toxicity, water quality objectives, acid deposition problems and state of the art for acute and sublethal toxicity testing. In addition, papers presented in the three concurrent workshops dealt with new techniques in toxicology, biochemical methods for toxicity evaluation and the use of invertebrates in toxicity. Synopses of the workshop sessions summarized by the session chairmen are included. Finally, various aquatic toxicity research programs across Canada are provided.

Key words: Environmental law, waste treatment, water quality objectives, aquatic, toxicology, bioassay, invertebrate testing, acid deposition, toxicity research

RÉSUMÉ DE COMPTE RENDU

Le compte rendu du cinquième atelier annuel de toxicologie aquatique comporte 26 articles, 6 résumés, une liste des programmes de recherches sur la toxicité menés au Canada, ainsi que les délibérations des trois séances en atelier.

Les travaux portaient sur le droit environnemental, les effets du traitement des déchets sur la toxicité, les objectifs relatifs à la qualité de l'eau, les problèmes liés aux dépôts de produits acides et les connaissances actuelles quant aux essais sur la toxicité aigüe et sublétale. De plus, les exposés présentés dans trois ateliers différents traitaient de nouvelles techniques de toxicologie, de méthodes biochimiques pour évaluer la toxicité et de l'utilisation d'invertébrés dans le domain de la toxicologie. Le compte rendu contient également les résumés des séances d'atelier rédigés par les présidents. On y trouve enfin divers programmes de recherches de toxicologie aquatique au Canada.

Mots-clés: Droit environnemental, traitement des déchets, objectifs de qualité de l'eau, toxicologie aquatique, bio-essai, expériences avec invertébrés, dépôt de produits acides, recherche sur la toxicité

Preface

This volume is the proceedings of the fifth in a series of annual workshops begun in Winnipeg in 1974 that have provided a forum for:

(a) discussions of issues relevant to aquatic toxicology;

(b) descriptions of new concepts, approaches and methodology;

(c) critical review of "the state of the art";

(d) presentation of new results of monitoring and research on toxic substances;

and (e) contacts among participants from universities, industries and federal and provincial governments.

The workshop is not sponsored by any formal scientific society or organization. Rather, each meeting has been organized and run by different volunteer groups, with funding coming from the participants and the agency employing the volunteer group. Consequently, the specific objectives, presentation and character of each meeting have reflected the interests of the local organizing group rather than of an umbrella society. In all meetings, however, there has been an obvious attempt to keep the discussions relevant to toxicological problems associated with protecting aquatic ecosystems.

The proceedings of past meetings may still be available under the following titles:

1. Freshwater Institute. 1974. Compendium of aquatic toxicity studies in Canada. Unpublished Report Fisheries and Environment Canada, Freshwater Institute, 501 University Crescent, Winnipeg, Manitoba.

2. Craig, G.R. 1976. Proceedings of the Second Annual Aquatic Toxicity Workshop, November 4-5, 1975, Rexdale, Ontario. Ontario Ministry of the Environment, Box 213, Rexdale, Ontario.

3. Parker, W.R., E. Pessah, P.G. Wells, G.F. Westlake. 1977. Proceedings of the Third Annual Aquatic Toxicity Workshop, November 2-3, Halifax, Nova Scotia, EPS Technical Report EPS-5-AR-77-1, Fisheries and Environment Canada, Environmental Protection Service, 5151 George Street, Halifax, Nova Scotia B3J 1M5.

4. Davis, J.C., G.L. Greer and I.K. Birtwell. 1978. Proceedings of the Fourth Annual Aquatic Toxicity Workshop, November 8-10, 1977, Vancouver, British Columbia. F & MS Technical Report 818, Fisheries and Environment Canada, Pacific Environment Institute, 4160 Marine Drive, West Vancouver, British Columbia V7V 1N6.

The Sixth Annual Aquatic Toxicity Workshop will be held at Winnipeg once again. Details are available from

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Editors' Comments

The proceedings of the Annual Aquatic Toxicity Workshop have grown in volume since their inception in 1974. This year's proceedings contain 26 papers and 6 abstracts all of which have been reviewed by referees. We wish to thank all referees for their constructive comments. As in the past proceedings, we did not exercise extensive editorial control on the paper format and style of presentation. However, we have standardized the first page of the paper with author's name, title of the paper and the proceedings number at the top and the author's address at the bottom of the page for easy reference and abstraction.

The Editors.

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The Editors.

Papers Presented (Cont'd) Page No.

16. Craig, G. R. and G. L. Beggs. An evaluation of loading rates in regulatory static bioassays. 145

17. Holt, J. D., and S. A. Malcolm. Threshold estimation in aquatic toxicity experiments (abstract). 161

18. Dixon, G. Acclimation to toxicants by rainbow trout and its potential use in predicting safe levels (abstract). 162

19. Hammond, B. and J. R. Bishop, Jr. The physiological evaluation of ozone and chlorine toxicity on channel catfish, Ictalurus punctatus. 167

20. Murphy, J. A., A. D. Smith, G. W. Ozburn and D. E. Orr. The acute and chronic effect of trichlorobenzene on discrete life stages of the American flagfish Jordanella floridae (Goode and Bean). 183

21. Kwasniewska, K., D. Liu and W. J. Strachan. Bioassay of relative toxicity of some chlorophenols using sewage bacteria. 193

22. Scherer, E., and S. Harrison. Locomotor activity testing using sonar beam interruptions. 201

23. Beckett, A. L., and B. T. Thackeray. A recycle holding system for freshwater and marine fish stocks. 211

24. Mayfield, C. I., W. E. Inniss and J. E. Thompson. Bioassay techniques for toxicological studies on aquatic organisms. 225

25. Morgan, R. P. Biochemical parameters as pollution indicators in fishes. 232

26. Ivanovici, A. M. Adenylate energy charge: Potential value as a tool for rapid determination of toxicity effects (no presentation; paper only). 241

27. Addison, R. F. Induction of hepatic mixed function oxidases in fish - a potential index of sublethal stress (abstract). 256

28. Malley, D. F., W. Dentry and S. L. Leonhard. Calcium influx into post-moult Daphnia magna: A potential sublethal toxicity test. 257

29. Friesen, M. K. Toxicity testing using eggs of the burrowing mayfly Hexagenia rigida (Ephemeroptera = Ephemeridae), with methoxychlor as toxicant. 266

Papers Presented (Cont'd) Page No

30. Chapman, P. M., L. M. Churchland, P. Thompson and E. Michnowsky. Tubificid oligochaetes as monitors of heavy metal pollution. 278

31. McLeese, D. W., D. S. Pezzack and C. D. Metcalfe. Uptake of PCB's from sandy sediments by *Nereis virens* and *Crangon septemspinosa*. 295

32. Lee, K., C. Nalewajko and T. R. Jack. Effects of vanadium on freshwater algae. (paper in poster session) 297

Appendix I - Discussion Workshop

Workshop 1 - New techniques in toxicology.
Chairman: G. Craig 311

Workshop 2 - Biochemical methods for toxicity evaluation.
Chairman: J. Klaverkamp 313

Workshop 3 - Invertebrate toxicology.
Chairman: B. Wilson 315

Appendix II - Aquatic toxicity research in Canada 316

Summarized by P. V. Hodson.

Appendix III - List of participants and addresses 335

Johnson*, M.G. 1979. Toxicology and Resources Policy - a means in search of an end. Proc. Fifth Annual Aquatic Toxicity Workshop, Hamilton, Ontario, Nov. 7-9, 1978. Fish. Mar. Serv. Tech. Rep. 862, pp. 1-14

Keynote Address

A substantial majority of the participants at this workshop are employed by fisheries management and environmental protection agencies. Most of you work on aquatic organisms, of one kind or another, for two dominant reasons beyond personal interest - freshwater communities support certain species of direct commercial and recreational value, and these aquatic communities are a barometer of the state of the ecosystem and hence reflect man's health and wellbeing. Most of you probably have served on one or more committees set up to establish criteria or specific water quality objectives for a lake or river system. You are concerned, therefore, about the extent of integration of toxicological findings in strategies for resources and environmental management, and how you should direct your own efforts in influential ways.

The Great Lakes and their basin form one ecosystem of many in which problems attributable to toxic substances pose constraints, sometimes extremely severe, on resource management goals. I propose to discuss some of these issues in the programs and the kinds of strategies which should be developed to deal with these issues, including toxicological research.

The Great Lakes System is home to 37 million people, 33% of the population of Canada and 14% of the population of the United States. The population is expected to rise to 60 million people by 2020; industrial and municipal growth is expected to rise accordingly (Pollution from Land Use Activities Reference Group, 1978). A significant majority of this

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population employs a Great Lakes water supply. The issue of long-term safety of these water supplies has not, to my knowledge, been addressed explicitly and formally by public health experts as an issue of international concern. Obviously this question has been implicit in water quality investigations generated under the auspices of the International Joint Commission; however, the participation by public health experts traditionally has been minimal. The subject, as important as it is, falls outside the field of expertise and mandate of most of us here. Consequently, I should move on to examine living aquatic resources, in particular fish.

Commercial fishing in Ontario produces the largest quantity of freshwater fish in Canada, with a gross value of 20 million dollars annually, mostly from the Great Lakes. With full and part-time employment for 5,000 people, including those engaged in processing, it is not large in terms of participants, although some Ontario Great Lakes port towns are primarily dependant on fishing. Recreational fishing in Ontario involves close to 3 million of the province's 8 million people and about two-thirds of a million non-resident anglers. In 1975 angling accounted for 40 million days of recreation and about 350 million dollars were spent by anglers on goods and services. The recreational catch was double to triple the commercial catch. In 1975, the U.S. Fish and Wildlife Service estimated that over 16 million angler-days were spent on U.S. waters of the Great Lakes; a large sum, estimated at 149 million dollars, was put into the economy.

Significant changes in fisheries of the Great Lakes are due partly to effects of pollutants, as well as to over-exploitation and introductions of exotic species such as smelt, alewife, sea lamprey and common carp. In 1969, with concern over rising DDT levels, 34 thousand pounds of coho salmon were confiscated in Michigan (Radonski, 1977). In March, 1970, Canada banned commercial sale of mercury contaminated fish in Lake St. Clair, the St. Clair River and Lake Erie. In April Michigan banned all fishing in Michigan waters of Lake St. Clair. Subsequently the ban was relaxed to permit catch-and-release fishing but few anglers were interested either in fishing for "poisoned" fish or in having to release their catches. For ten years now we have witnessed a series of commercial bans and recreational fishing advisories related to contamination by mercury, PCBs and, most recently, mirex. The mirex issue reached a climax on September 14, 1976, when the New York Commissioner of Environmental Conservation, on recommendation of the Commissioner of Health, issued a proclamation banning the possession of seven species of fish. NYECD biologists estimated that fishing pressure on the 1976 salmon run was down by 70%. The Ontario Ministry of Environment released a "Guide to Eating Ontario Sport Fish". Lake by lake, species by species, length class by length class, this book charts what fish should not be eaten by whom. In Lake St. Clair, for example, every species offers some degree of risk. Walleye, pike and largemouth bass pose highest risk. You are on safer ground by selecting catfish and carp. Bon apétit!

Dollar values alone will get attention, but for most people, sooner or later, there will be a deeper concern. Simply put, there will be a gut feeling, since fish and human wellbeing must be related in some ways, that strategies for resources and environmental management in the Great Lakes ecosystem are deficient in scope and/or application. In by-gone days miners used canaries to monitor their workaday ecosystem. The reaction to a dying canary was decisive and swift. Our actions to contaminant issues over the last decade generally seem akin to burying the canary and carrying on our workaday activities with least possible inconvenience and cost.

The focus on specific water quality objectives in the Great Lakes Water Quality Agreement of 1972 may be a useful short-term strategy in reversing deteriorating trends in the quality of boundary waters in the Great Lakes basin. However, it is important to understand the pitfalls associated with preoccupation with water quality objectives as a means to protect water quality and ecosystem integrity. Managing the quality of water alone is an inadequate tool to protect ecosystem health. We are overdue in developing a broader approach that takes account of interactions among the various components of the Great Lakes ecosystem as a whole.

The development of the specific water quality objectives, and their use as a management strategy, suffers the following limitations:

1. Sublethal chronic effects of mixtures of pollutants are not adequately considered.
2. Recycling of toxic substances from sediments directly to the biota via food chain uptake is ignored.

3. Poorly understood, problem substances are essentially uncontrolled.
4. Water quality objectives are curative, not preventative, as they are normally employed.
5. Water quality objectives are meant to be applied at boundaries of mixing zones which are seldom defined, not to mention inadequately monitored.
6. Dedication of incremental portions of nearshore habitat as mixing zones is a critical loss of a key portion of the ecosystem.
7. Pollutant loadings remain uncontrolled as continual increments of pollutant load could occur without violation of water quality objectives.
8. Physical habitat alterations are not taken into account. Processes such as wetland drainage, shoreline alteration, dredging, water level regulation and extractive uses of water all may affect the integrity of the aquatic ecosystem - but not by causing an alteration to water quality alone.

Most of these shortcomings were identified in the technical committee work under the Water Quality Board and IJC in which the objectives were developed and presented. However, it is apparent that these caveats tend to be forgotten or ignored. There is a tendency for water quality objectives to be portrayed by administrators, and held in the public mind, as adequate protectors of ecosystem integrity. It is true, that in the design of specific objectives, protection of "most sensitive use" is a fundamental principle. However, the objectives do not protect anything until applied, and when they are applied (at the boundary of mixing zones) the process in fact permits deterioration of environment and restriction of certain uses within mixing zones. It is a sad commentary that the only specific process in the 1972 Agreement geared to sustain a given use of the Great Lakes ecosystem is the process to parcel out and give away "mixing zones" to industries and municipalities to dilute wastes. "Mixing zone" is a physical term for a political consideration.

This approach is the main ingredient in the Great Lakes recipe for rehabilitation, and, in the words of the Water Quality Board "The objectives . . . form the basis for the water quality assessment and the remedial programs to control pollution" (GLWQB 1978). The Board goes on to say, "The assessment of available data and development of new and revised objectives was essentially completed with the WQB's 1976 Annual Report".

The front-line defence against adverse effects from toxic substances is that collection of preventive legislative which includes the Food and Drug Act, Pest Control Products Act, Hazardous Products Act and, as of December 2, 1975, when it received Royal Assent, the Environmental Contaminants Act. (There are U.S. equivalents - Federal Insecticide, Fungicide and Rodenticide Act, Food Drug and Cosmetic Act, and Toxic Substances Control Act.)

Where there are action guidelines, application of the Food and Drug Act to the commercial fish harvest is straightforward. Where there are no action guidelines we generally do not know the significance of residues because health agencies tend to react only when problem levels are suspected. This emphasis tends to lessen the value of fish contaminants monitoring as a preventive surveillance tool.

Progress in Canada under the Environmental Contaminants Act has been disappointing. The five regulations, in three years, include two on restricted uses of PCBs and three rather easy, painless bans (because the chemicals are not being used now in Canada) on polychlorinated terphenyls, polybrominated biphenyls and mirex. The main general problems appear to be lack of teeth in the Act and, probably because of that, some shortage of bureaucratic will power. Specifically, it appears that before the agencies can demand information on chemicals of concern, they must have more information than is likely to be available, just to provide the required basis of concern to legitimize the request. And, unlike the U.S. Toxic Substances Control Act, there is no mandatory reporting on new chemicals prior to manufacturing and/or importation.

Without inadvertently downplaying synthetic organic contaminants, where the obvious answer is to control their production and use there seems to be insufficient attention being paid to strategies to reduce toxic substances of long standing, the heavy metals. Kemp and Thomas (1976) found substantial enrichment of surficial lake sediments of Lakes Ontario, Erie and Huron (relative to the *Ambrosia* horizon 120 BP) with mercury, lead, zinc, cadmium and copper. Similar conclusions were reached for Lake Michigan (Leland *et al*., 1973) for mercury, lead, zinc, copper and chromium. Anthropogenic loading of heavy metals is low in Lake Huron and high in Lakes Erie and Ontario (about 10x for mercury, 4x for lead, 2x for zinc, for example, in Lake Erie; close to 50x for mercury, 10x for lead, 5x for zinc in Lake Ontario). The most recent review of trace element concentrations in Great Lakes offshore waters (PLUARG 1978) indicated that cadmium and copper were approaching so-called safe concentrations through most of the Great Lakes. This must be viewed with concern in the light of observations on combined effects (Wong *et al*. 1978) and projections of the likely inputs of metals to the lakes from atmospheric pollution (Acres Consulting Services Ltd., 1977). As you know, Wong and colleagues found that recommended safe levels of a number of metals (Great Lakes specific water quality objective levels) were very toxic to selected freshwater algae when presented simultaneously in lake water and culture medium.

Trace metals emissions to the atmosphere from point sources are expected to increase by 2.5 to 3.5 times by 2000. The lower rate is a pro-nuclear scenario, while the higher rate is expected if coal replaced planned nuclear facilities.

In addition, recent municipal waste management practice for phosphorus removal, much of which is done with metals-contaminated iron and aluminum salts, could aggravate the problem. In Ontario only 14% of this chemical sludge is deemed sufficiently low in metals for disposal on rural land. About 40% is incinerated and the balance goes to sanitary landfill sites. The degree of regulation, surveillance and enforcement of industrial waste by-laws by the municipalities also needs examination. It would not be a surprise to anyone, I am sure, if the optimum, cost-effective strategy consisted of control of metals at source, that is, in industrial plants, in household products and in the chemicals employed in phosphorus precipitation at sewage treatment plants.

Environmental assessment protocols form a less explicit, indirect approach to control toxic substances (for example, the Eldorado Nuclear plant proposals being examined under the federal Environmental Assessment and Review Program). These often tend to bog down in project detail, while the more fundamental policies and cumulative project effects are not adequately considered.

Where should we go from here? First we have to recognize that specific water quality objectives have limitations in application. As a reference point for interpretation of trend-in-time data on pollutant concentrations, the objectives can provide some sense of urgency and/or priorities among pollutants. They may be suitable for development of controls on individual polluters, but they appear to have limited value for planning in broader contexts. Loading controls on pollutants appear to be the most rational means at present to protect and enhance environmental quality. Loading controls, as they have been used for phosphorus input control to the Great Lakes, provide a tool for planning. Loading reductions for each lake are allocated between Canada and the United States and could be further allocated within each country. Although loading for persistent contaminants would have to be zero, for some toxic substances, e.g. heavy metals, loadings ceilings and reductions in inputs would have to be determined.

What we are looking at, therefore, is a tolerable dose, which must never be exceeded, at the ecosystem level. Comparing earlier pollutant loads and environmental quality at those earlier times and comparing conditions in a series of lakes with their respective loading rates are approaches for developing empirical relationships to prescribe reductions for desired improvements. This task is exceedingly more difficult than traditional toxicology. It is an "ecosystem epidemiology" which would lack all of the orderliness of lab experimentation. It would call for the close collaboration of physiologists, pathologists and limnologists. Not that controlled experimentation would not be required .....it certainly would ..... but hypotheses arrived at from field observations would be taken to the laboratory for testing. This is different than the present paradigm in which lab observations are taken to the field . That is, if there are any hypotheses taken to the field at all! Many results go direct to the boardroom and find their way into vast tables of criteria, objectives and standards without the kinds of technical qualification mentioned earlier and often without a sound conceptual basis (in resource or environmental management) for their application.

It is suggested, in the draft 1978 Agreement on Water Quality in the Great Lakes, that mixing zones be called "restricted use zones" on the basis that a spade deserves to be called a spade. We must consider these zones to be nothing more or less than zones of non-compliance by point-source discharges, where, in consideration of the tradeoffs among socio-economic factors and technological know-how, one or more pollutants are in violation of water quality objectives. The challenge in point-source wastewater management is either to overcome technological and economic constraints to achieve specified effluent objectives or to replace problem materials with safe alternatives.

The need to control toxic substances at source becomes more and more evident daily. Waste processing, handling and disposal problems are a bureaucratic nightmare. Information systems are taxed beyond the breaking point. Many decisions are so obviously expedient. For example, the OME decision of this past summer to allow up to 25 ppm PCBs in waste oil usage for road dust control was a poor decision in principle but alternatives, in the Ministry's opinion, may have been beyond their capabilities to administer effectively. It is essential that federal governments provide leadership and assistance in this critical area by vigorous application of preventive legislation. In Canada's case at least, this will require putting teeth into the Environmental Contaminants Act. This should automatically bring about the willpower now lacking in its application. It is in this area of supporting preventive controls that our toxicological studies will be most useful and rewarding. This is not just to rationalize existing approaches. There is still a need for broader approaches to examine potential hazard to ecosystems at various potential "dose" levels.

It is not clear how effective environmental assessment protocols are likely to be in control of toxic substances. In view of the fact that public participation is likely to be vigorously and effectively applied at this pragmatic level (as opposed to more abstract issues), it is conceivable that assessment hearings may bring about policy changes with broader implications than individual projects. In these cases, as in cases of environmental deterioration, there is an onus on resource and environmental agencies and affected parties to marshall a great body of evidence. Even if resources to do this are allocated, by the time that strong evidence has been obtained, the resource may have been so depreciated that the users have faded away and the only voice left may be that of the investigator himself. Obviously, we have to press for a number of changes. We should plan to do more complete work at fewer sites to establish the sound knowledge for broader application, to respond firmly on the basis of inductive reasoning, and to act quickly while there are still affected parties with standing to make use of the information. Funding levels for such work are inadequate at present and should be increased.

In closing, I would like to refer to some important, closely related aspects of current value systems which need to be addressed. We should do whatever we can to "depolarize" the issue of environmental versus socio-economic benefits. Environmental benefits are now poorly recognized, largely unquantified in any useful terms and, as a substitute, heavy emphasis is placed on surrogate technical goals (ppm, tons, etc.). These objectives are in fact part of our methodology rather than social goals. We simply must seek recognition for environmental benefits as socio-economic benefits in both the short and long term. My second point is that there should be resource rent for free open-access aquatic resources (a dividend to the public, the stockholders of the resources). This is needed not so much to raise revenue per se, but rather to place a value on the resource to society as represented by that revenue. The right to pollute, that is to use public waterways to dilute wastewaters should not be free. In fact, it should be so far from bargain rates that it is a solid inducement to reduce pollutant discharges. Royalties are needed in particular in recreational fisheries. Resident licences are not now required in Ontario. Resource rent or royalties would be important also, as a matter of principle, in commercial fisheries. Free, open access now places aquatic resources in a second rate relationship with other valued resources. We would be on much stronger ground if we could argue in explicit socially relevant terms in defence of a resource that is recognized as a public asset. In due course, the management success and wellbeing of that resource will be considered as a measure of man's own success and wellbeing.

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INTRODUCTION:

As a lawyer working in the field of environmental litigation, I have had occasion to call aquatic biologists as expert witnesses in hearings and during trials. In some of these cases these biologists have conducted toxicity tests on samples of a substance discharged into a body of water.

I have found that the problems arising from the use of such evidence are not concerned with the validity of the test protocols or their results which goes to the weight of the testimony but with the admissibility and relevance of this evidence in relation to the issue in the case. The portion of the paper dealing with admissibility applies to the use of toxicity test evidence in all litigous proceedings including prosecutions under The Fisheries Act. The portion of the paper dealing with relevance applies specifically to prosecutions under the sections of the Act dealt with.

Evidence which is inadmissible will not be heard by the court at all. Evidence which is not relevant at all is also inadmissible, but where it does appear to bear on some matter in issue in the case the court will normally hear it before making a final ruling on its relevance. The weight of testimony relates to the degree of reliance that the court will place upon it on deciding a matter in issue. A court will reject evidence to which it attaches little weight by saying that it is insufficient to prove the fact in issue (in a prosecution) beyond a reasonable doubt. An example of evidence relating to a toxicity test that would be given little weight is a test carried out in water that was too warm for the species tested and without controls.

Evidence of the results of a toxicity test and the proper conclusions to be drawn from them is admissible under 4 conditions:

1. ordinary people, if unassisted by persons with special knowledge, are unlikely to form a correct judgement:
2. the witness has special knowledge by study or experience;
3. the material tested is a sample of the same material that was discharged into a body of water;
4. the sample is in the same condition when it is tested as when it was collected.

Problems with admissibility are most often encountered in respect of the third and fourth conditions which, for this reason, will be dealt with in some detail in this paper.

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In order to be relevant, evidence must bear upon some matter in issue in the case. The precise issue is determined by the words of the statute which creates the offence. Mere reporting of the results of a toxicity test may not be sufficient for this purpose. This is therefore another problem area which will also be dealt with in some detail in this paper.

ADMISSIBILITY OF TOXICITY TESTS:

Lawyers and judges often refer to proof that the material tested is the same material as that which was discharged and that it is in the same condition when tested as when discharged as proof of "continuity of possession" of the sample. This is a misleading term as it implies that an unbroken chain of possession from the time of collection of the sample to the time of testing must be proven in every case.

Ideally, an unbroken chain of possession should be proven so that any problems in respect of the admissibility of test results are avoided. This can be done by carrying out the following procedures:

1. Sample is placed in sealed container immediately after it is taken (adhesive tape will do for a seal).
2. The container is marked for identification giving the sample number, date and time of sampling, name of sampler, type of sample, name and location of company or person responsible for discharge and sampling point.
3. Place sealed container in a locked box.
4. Personally deliver locked box to the person who will conduct the test or send the box by freight and the key separately by registered mail to that person.
5. Have the sampler initial the identification label and date it on receipt.
6. If a certificate is to be used, be sure that it fully identifies the same, recording all of the data on the identification label, as well as a statement setting out how and when the locked box was received, how and when the key was received and whether the sample was still in the sealed container and apparently undisturbed when the box was opened, then the results of the test.

In most cases, heavy reliance will not be placed upon this certificate as it will be necessary to call the aquatic biologist to testify as to the significance of these results and relate the results to the specific statutory prohibition. It is, however, useful to prepare the certificate at the time of the testing as a contemporaneous record of the essential facts necessary to establish that it is admissible. You can then consult the certificate to refresh your memory when the case comes to court many months later.

In many cases, the "infallible" procedures already outlined cannot be carried out. The most common circumstance arises from the fact that in most cases it is helpful to have an analysis of the material collected so that the aquatic biologist can also testify as to what chemical properties of the material were responsible for the lethality. Therefore, the material will pass through two or more sections of the laboratory where different analyses or tests will be carried out.

In this case the first person to receive the sample should, after removing an aliquot and preparing the certificate, deliver the remainder of the material directly to the person who will carry out the next test. This person should prepare his own certificate, setting out, in addition to the other matters, that the sample was delivered by a named person on a certain date, at a certain time.

Problems can also arise in large labs where the organization requires that samples be directed to section heads who assign the work to group leaders, who in turn supervise the technicians who carry it out. In this case the seal should not be broken until it is delivered to the person who will conduct the analysis.

Where samples cannot be analysed or tested shortly after receipt, it is desirable that they be kept in a room or container which is kept locked when staff are absent to exclude the possibility of tampering.

Generally speaking the courts are sensible and flexible in considering the admissibility of test results as can be seen in R.V. Kilgore [1] where the admissibility of an analysis of a blood sample for alcohol in an impaired driving case was challenged. The accused had been taken to a doctor's office where the sample was taken and placed in a sealed tube. The policeman kept the tube in his home refrigerator over the weekend and then sent it to the crime lab by registered post. At the lab the analyst received the sealed tube in the outer envelope which had been opened. At the lab, the envelope has passed through the hands of at least one unidentified person.

The certificate of analysis set out the following information:

> "That on the 30th day of April, 1968, at Regina in the Province of Saskatchewan, there was produced to me a brown envelope which bore the following markings:
>
> '(a) On the envelope "R no. 808 Nipawin. Sask."
>
> '(b) On a sealed tube of blood within the envelope "9.15 p.m. 27 R. Kilgore: R. Kilgore 27-4-68 (a signature) M.D."
>
> 'That I marked the said tube of blood "469-68-A D.L.W."
>
> 'That I performed a chemical analysis on the blood within the said tube, and found it to contain 0.13% w/v ethyl alcohol."

The Court held that the sample was admissible giving the following reasons:

> "The fact that the chain of continuity of possession had been broken does not of itself render an article of evidence inadmissible: Regina v. Donald (1958) 28 C.R. 206, 41 M.P.R. 127, 121 C.C.C. 304. And when there is evidence that reasonably leads only to the conclusion that a sample was in the same condition when it reached an analyst, as it was when it was dispatched for analysis, and there is no evidence to suggest that it has been tampered with in the interval, such sample, and the results of the analysis, are admissible in evidence: Rex v. Kolkiczka (1933) 1W.W.R. 299."

More recently another court in R. v. McIlwaine 2 dealt with the question of the speculative possibility of tampering with a sample locked in a police cupboard in a drug case making these comments:

> "The likelihood or probability of an exhibit in which there is some hiatus in continuity evidence having been tampered with or changed is something that the Court looks at. And of course, the opportunity to tamper with an exhibit or to have the exhibit change its nature through any cause is something the Court looks at. But in this case, if there was any opportunity, any possible opportunity, that doesn't mean that the weight of the exhibit itself has been affected. For instance, it's possible that someone broke into the RCMP quarters in the night, some night in question, unlocked the door to the locker room, unlocked the locker, and substituted another package for the one that was there. Those are the possibilities, but we deal in probabilities, not in possibilities. There are all sorts of things that are possible. And no one has to sit on an exhibit or take it to bed with them. The fact that it is in a locked locker, you know, you could argue that well, it isn't in the presence of somebody. And people do take things out of lockers that are much stronger than police cupboards. But there must be evidence of probability of having been changed."

In most of the reported cases, the samples following analysis, ha been returned by regular mail, with the certificates, to the investig While the courts have commented that this is a proper procedure to fo I have been unable to find any case where the court has specifically sidered the question of whether it is essential that the sample be r for identification and comparison with the information on the certif in court.

In toxicity tests the sample is generally large in quantity a one sense used up or altered in the course of the conduct of the being emptied into a series of containers and diluted and having duced into it. In a recent prosecution against Cyanamid, the Co the testimony of the aquatic biologist without production of any portion of the sample or its container and I can see no reason in these circumstances why it should have to be returned for production in Court. No objection was, however, specifically made on this ground and it is, therefore, not absolutely certain how the Court would have ruled if it had been.

It may be advisable to keep a small portion of the sample in the original container and return it re-sealed to the investigator with a certificate of analysis after the testing has been completed.

RELEVANCE OF TOXICITY TESTS:

Toxicity tests are most likely to be used in the Province of Ontario in prosecution under S14(1) (a) of The Environmental Protection Act, 1971 and S32(1) of The Ontario Water Resources Act.

As indicated earlier, it is essential to remember in preparing your testimony that it should be directed towards proving the primary issue in the case. The precise issue is defined by the wording of the Act itself.

The Ontario Water Resources Act, section 32(1) provides as follows:

> "Every municipality or person that discharges or deposits or causes or permits the discharge or deposit of any material of any kind into or in any well, lake, river, pond, spring, stream, reservoir or other water or watercourse or on any shore or bank thereof, or into or in any place that may impair the quality of the water of any well, lake, river, pond, spring, stream, reservoir or other water or watercourse is guilty of an offence . . . " [3]

The primary issue from the point of view of the aquatic biologist is the issue of whether the material discharged into the water was such as "may impair" the quality of that water.

Evidence that fish died when placed in a sample of the material discharged is evidence from which the Court may infer that the material discharged was such as "may impair" the quality of the water. The Court may or may not draw this inference depending on the circumstances surrounding the discharge and the supplementary testimony which the aquatic biologist is able to give to assist it in drawing that conclusion.

In order to give that assistance, the biologist needs additional information about the chemical properties of the material and the quality of the water into which the material is being discharged. The biologist should be able to explain on the basis of a chemical analysis of the sample identifying the materials and their concentrations which materials are present in sufficient concentrations to kill the fish. He should also be prepared to comment on the additive, or synergistic or beneficial effects of the presence of one or more materials. Where the biologist has no direct experience with the effects of these materials he can testify on the basis of his review and assessment of the relevant literature.

Where other materials are known to be present in the water from other discharges, an analysis for identification and quantification of these materia should also be carried out. The biologist should also be prepared to testify as to the effects of the interaction between the material discharged and the receiving water on its viability as a medium for the support of aquatic life. It is conceivable that an alkaline discharge into an acidic body of water migh improve rather than impair its quality as a medium for the support of aquatic life. In such a case, the Court might not infer that the discharge was such as "may impair" the quality of the water even though fish died when introducec into the alkaline material. Should the acid discharge later be discontinued, the Court might then convict.

In a recent prosecution against Cyanamid a charge was dismissed where a Court refused to infer that the material discharged was such as may impair the quality of the water. While it was clear from the reasons given that the Court had failed to fully apprehend the evidence of the aquatic biologist, the case does illustrate that evidence that fish died when introduced into the material discharged is not in itself sufficient to establish that the quality of the water may be impaired by the discharge of the material. An appeal has been argued but no decision has yet been rendered.

In this case 88,000 lbs. of sulfinol was spilled along a railway siding, ran into a ditch draining into the plant sewer system and discharged into a creek emptying into the Welland River. The spill was not reported for 36 hours by which time the bulk of the material had already left the area. Some sulfinol still remained in the ditch from which it could be seen to discharge into the plant sewer system.

An inspector was able to collect a small quantity of the material spilled from the ditch for toxicity testing and chemical analysis. Chemical analysis revealed that the substance was highly alkaline. In the opinion of the aquatic biologist, the alkalinity caused the fish to die.

Previous studies on water quality in the creek established that it was already incapable of supporting aquatic life due to high concentrations of ammonia in material being discharged from the plant sewers. The biologist then went on to testify that adding an alkaline material to an ammonia laden material would increase the toxicity of the ammonia. In the circumstances of the case, no quantification of the degree of additional impairment could be made.

The Trial Judge apparently felt that the water could not become impaired by the sulfinol discharge because it was already impaired.

In other cases, the Courts have held that the term "may impair" means "has the capacity to make worse" [4] and in my view, the testimony of the biologist that sulfinol was toxic due to its alkalinity, and that it would increase the toxicity of the ammonia in the Creek water was evidence that the material discharged was such as "may impair" or "has the capacity to make worse" the quality of the Creek and River.

While it is true that in one old English case dealing with a similar statute that the Court held that the legal maxim "de minimus non curat lex" or "the law does not concern itself with trifles" [5] applies, it also refused to apply the maxim in a case where raw sewage was being discharged into a river already so contaminated by upstream users that water quality was actually better downstream than upstream of the sewage discharge. In assessing whether the matter was trifling, the Court looked to the nature and quantity of the material discharged rather than the degree of further impairment.

> The E.P.A., 1971 S14(1) (a) provides " . . . no person shall . . . discharge a contaminant . . . into the natural environment that,
>
> (a) causes or is likely to cause impairment of the quality of the natural environment for any use that can be made of it; [6]

Although this section could be used when a contaminant which is toxic to fish is discharged into a body of water, preference is normally given to S32(1) of the OWRA because of the lesser burden of proof -- "may impair" as opposed to "causes or likely to cause impairment". This section is, however, viable when a fish kill has occurred and toxicity testing of the effluent has been carried out to prove that it was the contaminant discharged that caused the fish kill. Even in these circumstances the aquatic biologist should have a chemical analysis of the contaminant available to him for the purpose of identifying what caused the fish to die and background data on water quality to determine whether the presence of some other contaminant could also have caused the fish kill.

It would be convenient in all cases to conclude the testimony of the aquatic biologist by asking him directly for his opinion on the ultimate issue for instance, in a prosecution under S32(1) of The OWRA the final question and answer would be the following:

Q. In your opinion, was the discharge of sulfinol into Miller's Creek a discharge of material that may impair the quality of the water in that Creek?

A. Yes.

Traditionally, the courts have refused to allow experts to state their opinions on the ultimate issue, although in recent years this rule has often been ignored. In order to avoid this difficulty, you may be asked a question which does constitute an opinion on the ultimate issue but paraphrases the words of the statute. For instance, in the example already given the question could be altered as follows:

Q. In your opinion, was the discharge of sulfinol into Miller's Creek a discharge of material that had the capacity to worsen the quality of the water in that Creek?

A. Yes.

As the courts have held that "may impair" means "has the capacity to make worse" you have done indirectly, something which a court might or might not permit you to do directly, without flagging a ground for objection by opposing counsel.

In most cases it is to be hoped that an affirmative answer to this question will be obvious and compelling on the basis of the facts proven and the opinion given as to their significance, whether the "ultimate" question is posed and an answer allowed to be given, or not.

FOOTNOTES

1. R. KILGORE (1969) 6 C.R.B.S. 264
2. R. MCILWAINE, SUPREME COURT OF ALBERTA, OUGLEY, J. May 9, 1978, unreported.
3. THE E.P.A. 1971, S.O. 1971, Ch. 86
   R. CYANAMID OF CANADA LIMITED, PROVINCIAL COURT (CRIMINAL DIVISION) for the Judicial District of Niagara South, Dec. 15, 1977, unreported.
4. R. v. SHERIDAN (1973) 2 O.R. 192
   R. v. CHEROKEE DISPOSALS AND CONSTRUCTION LTD. (1973) 3 O.R. 599.
5. STAFFORDSHIRE CO. AND SEISDON RURAL DISTRICT CO.
   (1907) KNIGHTS LOCAL GOVERNMENT REPORTS 347.
6. THE O.W.R.A. R.S.A., 1970, Ch. 332.

Garrett[*], C., O.E. Langar[*] and G. Hebert[*]. 1979. Proving Toxicity in Court Without Bioassays. Proc. Fifth Annual Aquatic Toxicity Workshop, Hamilton, Ont., Nov. 7-9, 1978. Fish. Mar. Serv. Tech. Rep. 862, pp. 23-31

In January, 1977, a faulty transformer at the Canadian Cellulose pulp and paper mill near Prince Rupert, B.C., malfunctioned, resulting in the entry of approximately 800 litres of PCB fluid into the adjacent receiving waters of Porpoise Harbour, B.C. Environmental monitoring programs indicated that the harbour sediments in the immediate vicinity of the spill, contained excessively high levels of contamination. Consequently, charges were laid by the Environmental Protection Service under Section 33 (2) of the Fisheries Act.

This presentation will describe the methods employed by the Crown to prove deleteriousness of PCB's. Due to the difficulty in establishing chronic sublethal effects of contaminants such as PCB's in short-term laboratory studies, the procedure of presenting data obtained through bioassay testing was not employed in this instance. Deleteriousness was proven successfully by expert witness testimony based on toxicity information obtained from published literature.

This case, as well as many other Fisheries Act, Section 33 (2) prosecutions, demonstrates that in many situations deleteriousness of chemical contaminants, can be proven quite satisfactorily by a "common sense" approach thus negating the necessity of performing complicated bioassays involving procedures that can be criticized by defence experts in court.

INTRODUCTION

This paper will outline events relating to the successful Fisheries Act prosecution of a toxic chemical spill (PCB's) in which deleteriousness was proven without relying on the use of bioassays. It will further demonstrate that when the general nature and toxicological properties of a substance or class of substances is well documented in the literature, it is not necessary to resort to bioassay tests to prove deleteriousness to aquatic life.

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Experience obtained during past Pacific Region Fisheries Act prosecutions indicates that in certain situations, bioassays may be a greater handicap than an aid to successful prosecutions due to the various criticisms that can be made by laboratory toxicity tests.

CASE STUDY - PCB SPILL AT CANADIAN CELLULOSE

In January, 1977, a transformer at the Canadian Cellulose pulp and paper mill near Prince Rupert, B.C., malfunctioned and deposited approximately 800 litres of PCB fluid into the storm sewer system and consequently into the marine waters of Porpoise Harbour. The initial monitoring program indicated that sediments in the immediate vicinity of the spill contained excessively high levels of contaminat Analyses showed that levels of up to 8000 ppm were detected in sedime collected directly off the storm sewer outfall. The Environmental Protection Service subsequently charged the Company under Subsection of the Fisheries Act as it applied prior to its September 1977 amendm

Prosecution Under the Federal Fisheries Act

Section 33 of the Fisheries Act is the most widely used piece of aquatic pollution control legislation in Canada. Subsection 33(2) is concerned with the deposit of deleterious substances. It is administered federally by the Environmental Protection Service but is also applied by several provincial agencies in Canada. This subsecti has been used in more prosecutions in British Columbia than in all oth provinces combined.

Subsection 33(2) states:

"Subject to subsection (4), no person shall deposit or permit the deposit of a deleterious substance of any type in water frequented by fish or in any place under any conditions where such deleterious substance or any other deleterious substance that results from the deposit of such deleterious substance may enter any such water".

Expert witness testimony for the Crown stressed three major characteristics of PCBs which make their release to the environment of great concern:

1.) persistence in the environment
2.) tendency for biomagnification through the aquatic food chain
3.) toxicity to aquatic organisms at sublethal concentrations

In order to foil attempts by the Defence to convince the Court that bioassays were necessary to scientifically prove deleteriousness testimony of Crown expert witnesses emphasized that the deleterious effects of PCB's could not be readily demonstrated through laboratory toxicity tests.

The Crown's position in this respect was supported by the judge who stated that he "was satisfied that ..."

1.)"it was not possible to conduct the types of tests" necessary to show:
    a.) full life cycle effects
    b.) body burdens
    c.) sublethal effects
    d.) food chain buildup
2.) he "could rely on the opinions of the experts" and their "readings" of the opinions of other experts

Although the Defence did conduct bioassays the Court found the results of these tests to be "inconclusive" and as "there was too much emphasis on mortality rate rather than the sub-lethal effects" they did not prove non-deleteriousness.

Litigation Outcome

On March 14, 1978 Canadian Cellulose was found guilty of depositing a deleterious substance (PCBs) in a place under conditions where it may enter water frequented by fish and was fined $3500 per day for a period of seven days. Since the spill occurred prior to the September, 1977 Fisheries Act Amendments the maximum fine was $5000 per day.

Violations under Subsection 33(2) are now subject to a maximum fine of $50,000 for the first offence and $100,000 per count for subsequent offences.

This was a precedent setting case in that it was the first time that PCBs were shown to be a deleterious substance under the Fisheries Act. However, considering the vast amount of available information relating to the toxicity of PCBs to aquatic organisms, we believe that it would have been 'next to impossible' to prove PCBs to be non-deleterious, with or without the use of bioassays.

OTHER PACIFIC REGION FISHERIES ACT PROSECUTIONS

The Environmental Protection Service and Fisheries and Marine Service in the Pacific Region and the British Columbia Fish & Wildlife Branch have been very successful in proving the deleteriousness of several other substances under Subsection 33(2) of the Fisheries Act. These substances include mine waste and tailings silt, coal fines, oils and greases, gasoline, fuel oils, certain oil dispersant chemicals, sawdust, copper sulphate, and pentachlorophenol.

In the majority of cases, deleteriousness was established without conducting toxicity tests.

Our general philosophy with respect to prosecutions has been, "If a substance is generally known to be toxic or deleterious to fish life, why try to demonstrate this for each court case by conducting bioassays that often have severe limitations?"

For the purposes of the Act, the term 'deleterious substance' is defined in Subsection 33(11) as;

"(a) any substance that, if added to any water would degrade or alter or form part of a process of degradation or alteration of the quality of that water so that it is rendered deleterious to fish or to the use by man of fish that frequent that water,.... "

Amendments to the Act, effective September,1977, expanded the generality of the provisions of Subsection 33(11) by adding the words "likely to be rendered deleterious to fish or fish habitat" and also increased the fines under the Act.

Proving Deleteriousness in Court

Possible strategies for proving deleteriousness were reviewed by the lawyer and E.P.S. staff. Our experience indicated that there are three main strategies by which deleteriousness has been proven in court; 1) by introducting evidence relating to on-site observations of harmful effects on aquatic life; 2) by conducting bioassay tests to determine the direct toxicity of the substance in question to test organisms and 3) by the use of expert witness opinion testimony pertaining to the general deleterious effects of that substance on aquatic life.

The first method is the most obvious and least complicated and is used in instances of fish kills or where obvious stress effects, such as respiratory difficulties in fish,were documented on-site. However, as was expected, EPS officers investigating the PCB spill did not observe any such acute effects, other than those normally found in the vicinity of a pulp mill.

The approach of using bioassay tests to prove deleteriousness is used where regulations under subsection 33(4) of the Fisheries Act specify such or in prosecutions relating to a non-regulated discharge, and in instances where the general composition and toxicity of the effluent or other discharge is variable, unknown, or cannot be determined by chemical analysis.

Problems Encountered with Using Bioassays in Court

Unfortunately, toxicity tests have several shortcomings and if not presented in the proper context may actually handicap the Crown's case Some of the shortcomings of toxicity tests and consequent problems which may be associated with the introduction of bioassay results in court include;

1) bioassays cannot duplicate natural conditions of aquatic systems and consequently there are many influential factors which are not accounted for during laboratory toxicity tests
2) the deleterious effects of many contaminants do not lend themselves to evaluation by acute toxicity tests. Often it is the long-term and sublethal effects that are of major concern.
3) the introduction of bioassay results may necessitate that bioassay technician and backup expert appear in court to provide testimony regarding bioassay procedures and toxic effects
4) complicated bioassay techniques are difficult to explain to laymen and may add to confusion of the court
5) the more analytical techniques which are introduced the greater the opportunity for criticism by defence experts

Due to the difficulty in establishing chronic sublethal effects of contaminants such as PCBs in short-term laboratory studies, and because of the vast amount of available literature pertaining to the toxic effects of PCBs on aquatic organisms, the procedure of presenting data obtained through bioassay testing was not employed in the Canadian Cellulose prosecution. Deleteriousness was proven successfully by expert witness testimony based on toxicity information obtained from published literature.

Non-Chemical Pollutant - Silt/Sediment

When considering certain pollutants such as silt/sediment, routine toxicity tests are relatively useless in demonstrating deleterious effects. The hazardous effects of major silt releases differ substantially to those posed by chemical spills and cannot accurately be labelled as toxic effects. Silt is considered to be more of a 'physical' pollutant and its deleteriousness to salmonids is well documented in the literature. Once a deposit of noxious substance such as silt is proven, expert witness testimony must outline how this substance degrades water quality and renders it unfit for the survival of aquatic organisms. Testimony concerning the deleteriousness of silt to salmonid species includes a discussion of turbidity feeding impacts, loss of food sources, abrasive effects, habitat smothering, decreased survival rate for eggs and emerging alevins, and the physical clogging of gills. Few of these impacts can be meaningfully demonstrated through bioassays and for this reason bioassay results have never been introduced in Pacific Region silt prosecutions. It is interesting to note that attempts to prove the deleteriousness of silt by expert witness testimony has been successful in every case.

Surely, if it is possible to prove a naturally occurring substance such as silt/sediment to be deleterious, the task of proving the deleteriousness of PCBs and most other recognized pollutants, without resorting to legal bioassays, should be a far simpler task. Admittedly, if it was necessary to prove that the 'amount' of silt or material added to water created a deleterious situation, the issue of deleteriousness would be much more difficult to prove.

Fortunately, under the provisions of Subsection 33(2) it is necessary for the Crown to prove only that there was a deposit of a substance and that the substance in question is deleterious to fish or to the habitat of fish.

SUCCESS RATE OF PACIFIC REGION FISHERIES ACT PROSECUTIONS

Of forty-eight selected Fisheries Act Subsection 33(2) prosecutions in the Pacific Region outlined in the following table, legal bioassays have been used on only ten occasions. Of these ten cases, four (40%) were lost on the basis of bioassay information. Of the other thirty-eight cases, deleteriousness was proven successfully by expert witness testimony and guilty convictions were obtained on thirty-two cases (84%). The cases that were lost were usually lost on the basis of a legal technicality.

SELECTED FISHERIES ACT S
IN THE

SUBSTANCE	NUMB CA
Silt	2
Coal Fines/Silt	
Mine Waste and Tailings	
Log Conditioning Water	
Weak Black Liquor	
Sawdust	
Fuel Oil and Gasoline	
Oils/Greases	
Oil Dispersants	
Copper Sulphate	
Pentachlorophenol	
Polychlorinated Biphenyls	
Chlorinated Paint	
Concrete Plant Effluent	

UBSECTION 33 (2) PROSECUTIONS
PACIFIC REGION

ER OF SES	BIOASSAYS PERFORMED	NUMBER OF CONVICTIONS
21	No	16
1	No	1
5	No	5
1	Yes	-
1	Yes	-
1	No	1
9	No-7 Yes-2	9
2	Yes	2
2	Yes	1
1	Yes	1
1	No	1
1	No	1
1	Yes	-
1	yes	-

MacLatchy*, J. 1979. The role of toxicity testing is establishing that an effluent is a deleterious substance under Section 33 of the Fisheries Act. Proc. Fifth Annual Aquatic Toxicity Workshop, Hamilton, Ontario, Nov. 7-9, 1978. Fish. Mar. Serv. Tech. Rep. 862, pp. 32-45

In this paper the role of the acute lethality toxicity test in Section 33 of the Fisheries Act will be examined from two points of view:

(i) the use of the test in prosecutions to establish that an effluent is a deleterious substance, and

(ii) the development of regulations to prescribe a particular procedure for a toxicity test.

PROSECUTIONS

The Fisheries Act

Reference should first be made to the relevant subsections of the Fisheries Act which are found in the Appendix of this paper. Subsection 33(2) basically says that no person shall deposit a deleterious substance in water frequented by fish. Subsection 33(4) says that subsection 33(2) does not apply if the deposit of the deleterious substance is authorized (i) under regulations made under another federal statute, or (ii) under regulations made under subsection 33(13) of the Fisheries Act. Subsection 33(11) contains a definition of deleterious substance and subsections 33(12) and (13) provide for naming deleterious substances and for authorizing their deposit.

In this discussion it is most important to carefully examine the wording of the definition of deleterious substance in subsection 33(11).

Deleterious Substance

The phrase "deleterious substance" is used in both subsections 33(2) and 33(4) while the definition of deleterious substance is found in subsection 33(11). The definition of deleterious substance is rather long and is more readily understood if broken out into its individual components. Please refer to the definition as it is presented in the Appendix to this paper.

The definition of deleterious substance is comprised of two parts:

(i) general definitions which are contained in paragraphs (a) and (b), and

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(ii) specific definitions which are described in paragraphs (c), (d), and (e) and which are created by regulations under the authority of subsection 33(12).

Appearing between the general definitions of paragraphs (a) and (b) and the specific definitions of paragraphs (c), (d) and (e) are the following words:

"and without limiting the generality of the foregoing include"

Let us look at some of the practical problems involving pollution control so that the rationale for defining the words deleterious substance in this manner will become more apparent. The general portion of the definition of deleterious substance is not very definite for those who are not familiar with fish and toxicity. It would be preferable from their point of view if particular deleterious substances be named and the deposit of certain amounts of some deleterious substances be authorized. However, on the other hand there is no limit to the number of chemicals which are deleterious substances. Thus, it is impossible to make regulations that would define every possible deleterious substance and specifiy a quantity that may be deposited. Therefore, when Parliament enacted the definition of deleterious substance, Parliament defined a general definition with a provision that specific deleterious substances could be names "without limiting the generality of the" general definition.

The effect of defining deleterious substances has two particular benefits:

(i) it clarifies the subject for those concerned with particular deleterious substances, and

(ii) it eliminates the need to call evidence to prove that a particular substance is deleterious if it is specified in the regulations.

Paragraphs 33(11)(a) and (b)

The only real difference between pargraph 33(11)(a) and paragraph 33(11)(b) is that paragraph (a) covers the situation where a deleterious substance is deposited while paragraph (b) covers the situation where the deleterious substance is deposited with water as an effluent. For example, a deposit of solid cyanide would come within paragraph (a) while the same cyanide dissolved in a volume of water would be covered by paragraph (b). In the case of a pulp mill, the whole pulp mill effluent could be said to be the deleterious substance. On the other hand, the effluent may be regarded as particular deleterious substances that are being transported by natural water.

Paragraphs 33(11)(c), (d) and (e) and Toxicity Tests

While regulations have been made for various industrial sectors, only the Pulp and Paper Effluent Regulations prescribe "toxic wastes deposited by a mill" as a deleterious substance. In all other industrial sectors the toxicity test is only a guideline.

Subsection 3(2) of the Pulp and Paper Effluent Regulations defines "toxic waste" as any waste that is found to be toxic when tested in the manner described in Schedule D (See Appendix). You will note that this test calls for 35% dilution and more than 80% survival of fish in contrast to the more common standard of no dilution and more than 50% survival in 96 hours.

The Pulp and Paper Effluent Regulations do not create any offences. Failure to comply with the Regulations simply results in the Commission of an offence under subsection 33(2). The Pulp and Paper Effluent Regulations serve two legal functions:

(i) they define certain substances as deleterious substances, and

(ii) they authorize the deposit of certain deleterious substances under certain conditions for application to paragraph 33(4)(b).

The Irving Case has been the only prosecution where a toxicity test prescribed by regulation has been used. In all other cases the toxicity test has been used as simply one element of evidence to prove that the substance was deleterious in accordance with paragraphs (a) and (b) of the definition of deleterious substance.

The Receiving Water in Paragraphs 33(11) (a) and (b)

Particular notice should be given to the phrases:

(a) if added to any water...so that is is rendered deleterious, and

(b) if added to any other water...so that it is rendered deleterious

in paragraphs (a) and (b) respectively.

It should be noted that an acute lethality toxicity test on fish is a laboratory test, just like any other laboratory test that measures a particular parameter. The only difference between the test for acute lethality to fish and any other standard test is that the acute lethality test measures a biological parameter (death or survival of a living organism) while other tests usually measure a physical or chemical parameter (i.e. the temperature of a sample or the concentration of a metal or chemical in a sample). The test for acute lethality is a "white mouse" laboratory test. The legal connection between the test for acute lethality in the laboratory and the effect in the receiving body of water can be accomplished in two ways:

(i) introducing appropriate evidence, commonly an expert opinion of a biologist, or

(ii) using a test for acute lethality that is defined in regulations, such that any effluent that fails the test prescribed in regulations is defined as a deleterious substance. (Only applicable for new, expanded and altered pulp mills.)

Except in the case where the Pulp and Paper Effluent Regulations apply, it is essential that the Crown introduce expert evidence on how the failure of the toxicity test relates to a deleterious effect on fish in the receiving water. Without the proper connection to the receiving water by an expert witness, the toxicity test is useless.

It is most important that expert witnesses explain the function of the toxicity test. Many of the courts are reluctant to accept failure of the toxicity test as proof that effluent is deleterious without a great deal of explanation. To them it does not seem reasonable that fish should be expected to live in 100% effluent. It should be explained to the court that survival for 96 hours is a very short period of time. Many of the deleterious effects on fish can take place at very low concentrations over the whole life of the fish. If we are to protect fish, we must protect them over their whole life cycle not just 96 hours. If an effluent (particularly when there is a huge volume as in the case of a pulp mill) can kill fish in less than 96 hours (or in a much shorter time) such an effluent still may be objectionable if it is diluted 10, 100, 1,000 times or even more.

The court should be informed by expert witnesses that there are many industries that produce effluents where 100% of fish can survive the 96 hour test and even longer tests. The court should be made to understand that the Crown's test for 50% survival in 96 hours is a very reasonable test to expect the industry to meet.

Recent Amendments to the Fisheries Act

Bill 38, which is now known as Chapter 35, Statutes of Canada 1976-77, included an amendment to the definition of deleterious substance that broadened the application of paragraphs (a) and (b) in subsection 33(11). The words "so that it is rendered deleterious to fish" were expanded to read "so that it is rendered or is likely to be rendered deleterious to fish or fish habitat..."

It may be difficult for an expert to give an opinion with certainty that a particular water is rendered deleterious to fish. An expert may be more willing to give an opinion that a particular water is likely to be rendered deleterious to fish or fish habitat.

Case Law

The practical problems of the toxicity test in prosecutions may be best illustrated by informally discussing some of the following cases where toxicity test have been reviewed by the courts:

Cases Considering Toxicity Testing

Capt. J. Tindale, oil dispersants,
B.C. Prov. Ct., Johnson, J., Apr. 23, 1975,
Discussion of toxicity test at pages 4-7 of judgment.

B.C. Forest Products, oil dispersants,
B.C. County Court, Cashman, J., June 17, 1976,
Extensive discussion of deleterious substance on testing judgment.

MacMillan Bloedel, log conditioning water,
B.C. County Court, Proudfoot J., March 17, 1976,
County Court upholds Prov. Ct. decision.

West Coast Reduction, fats, oil emulsion,
B.C. Prov. Ct., Selbie, J., May 1, 1973,
Discussion on toxicity test at pages 4-6 of judgment.

Irving Pulp and Paper (Case 1), mill effluent,
N.B. Prov. Ct., George, J., Oct. 1, 1976,
Reference to toxicity test in Pulp and Paper Regulations.

Irving Pulp and Paper (Case 2), mill effluent,
N.B. Prov. Ct., Harrigan, J., Apr. 15, 1977,
Guilty plea, toxicity test according to Regulations.

Imperial Oil and B.C. Hydro, Oil spill,
B.C. County Court, Catliff, J., Nov. 20, 1975,
Discussion deleterious substance and tests at pages 9-10 of judgment.

Norman Kirby's gasoline spill,
B.C. Prov. Ct., May 8, 1972, Johnson, J.,
Discussion of toxicity tests at pages 6-7 of judgment.

Imperial Oil, oil spill,
N.S. Prov. Ct., Kinball, J., March 6, 1975,
General discussion of toxicity tests and deleterious substances.

Great Canadian Oil Sands, Tailings and seepage,
Alberta Prov. Ct., Aime, J., Feb. 23, 1977,
Discussion of toxicity tests on page 3 of judgment,
upheld on appeal to
Alberta District Court, McClung, J., Jan. 10, 1978,
Discussion of toxicity test on pages 4-10.

It is not necessary to always use the toxicity test to prove that something is a deleterious substance. In various cases expert opinions without toxicity tests were used in evidence to establish that substances were deleterious.

Expert Opinions Without Toxicity Test

Jack Cewe Ltd,
B.C. Prov. Ct., May 31, 1973,
Discussion of expert evidence on pages 2-3 of judgment.

Chew Excavating Ltd. and the District of Saanich,
B.C. Prov. Ct., Ostler, J., May 16, 1978,
Discussion of expert evidence on pages 7-8 of judgment.

Canadian Forest Products, oil spill,
B.C. Prov. Ct., April 14, 1978,
Discussion of expert evidence on pages 5-11 of judgment.

MacMillan Bloedel (Alberni) Ltd., oil spill,
B.C. County Court, McClellan, J., June 12, 1978,
Expert evidence on pages 6-11, supports Catliff, J. at page 4.

Canadian Cellulose (PCB spill),
B.C. Prov. Ct., March 14, 1978, Romilly, J.,
Discussion of expert evidence and absence of toxicity test at pages 93-102;
At page 96 the Judge rejects the B.C. Forest Product Case (Cashman J.) and the GCOS Case, and states that he perfers the judgment in Imperial Oil and B.C. Hydro Case (Catliff).

It should be noted that the decisions of the various courts on what evidence is sufficient to establish that a substance is deleterious has been very variable. It also should be noted that the issue of deleterious substances has only been reviewed by provincial court and county court judges. Of the cases listed above, only five of the cases have been reviewed by a county or district court. The balance of the cases have been considered before a provincial court. The Imperial Oil and B.C. Hydro case before Judge Catliff, County Court, represents the broadest interpretation of the definition of deleterious substance (page 10 of judgment). In contrast, Judge Cashman in the B.C. Forest Products case places a more restrictive interpretation on the definition of deleterious substance and places a relatively heavy evidentiary burden on the Crown. Lower courts are generally bound to follow the decisions of higher courts on questions of law. In British Columbia there are two county court decisions that interpret the definition quite different. Therefore, a provincial judge may simply chose to follow whichever interpretation he choses. Until the higher courts review the definition of deleterious substance, lower court judges will be free to interpret deleterious substance as each individual judge choses.

While the issue of deleterious substance was considered in the MacMillan Bloedel case on log conditioning water by Judge Proudfoot of the B.C. County Court, this case turned more on the evidence that was actually presented to the court rather than the courts' interpretation of the definition of deleterious substance. In the MacMillan Bloedel (Alberni) case involving an oil spill, Judge McClellan of the B.C. County Court chose to follow the broad interpretation of Judge Catliff. In contrast, in the Great Canadian Oil Sands case Judge McClung of the Alberta District Court chose to follow the narrower interpretation of Judge Cashman.

DEVELOPMENT OF REGULATIONS PRESCRIBING TOXICITY TESTS

The development of procedures to be specified in regulations for toxicity tests involves considerations that are somewhat different than the development of a procedure for a toxicity test for a particular scientific purpose. A regulatory test is to determine whether an effluent is on the pass or fail side of an arbitrary line. The procedure for a regulatory test should be as simple as possible. The inclusion of additional elements in the regulatory procedure may place an added burden on the Crown that is unnecessary in many circumstances. Where it appears to the biologist that additional elements should be imposed on the procedure specified in a regulation in order to make the particular test more scientifically valid, the biologist may chose to do so provided that the additional elements are not contrary to the basic procedure (i.e. increased number of test fish, or replica tests).

When E.P.S. is developing procedures for bioassay tests for either regulations, biologists will often criticize the proposed test because it is not accurate enough from the scientific point of view. However, a regulatory test is not attempting to make subtle scientific observation. The basic regulatory test simply establishes whether the effluent in under, or over, the arbitrary line that is defined by the regulatory test. The regulatory line may not be a sharp distinct line, but rather a broader grey line. The same comment may be made about BOD tests. The fact that the line is grey does not detract for the usefulness of the test from the legal point of view.

From the practical point of view, this is quite acceptable. Our primary regulatory concerns should be primarily directed at those effluents that are seriously toxic (i.e. where practically all the fish die). If an effluent is a border line case with respect to failing the toxicity test, then we should look at other steps to remedy the problem other than conducting a prosecution. (Assuming other deleterious substances, such as mercury or PCBs, which do not exhibit their deleterious effects in acute lethality toxicity test are not being considered). As an extreme example, in the Irving Pulp and Paper case, some of the effluents were so toxic that the first fish was dead before the next fish was placed in the effluent sample. In such circumstances it is of no importance whether it is a continuous flow through test, how many fish are used or what control test is used.

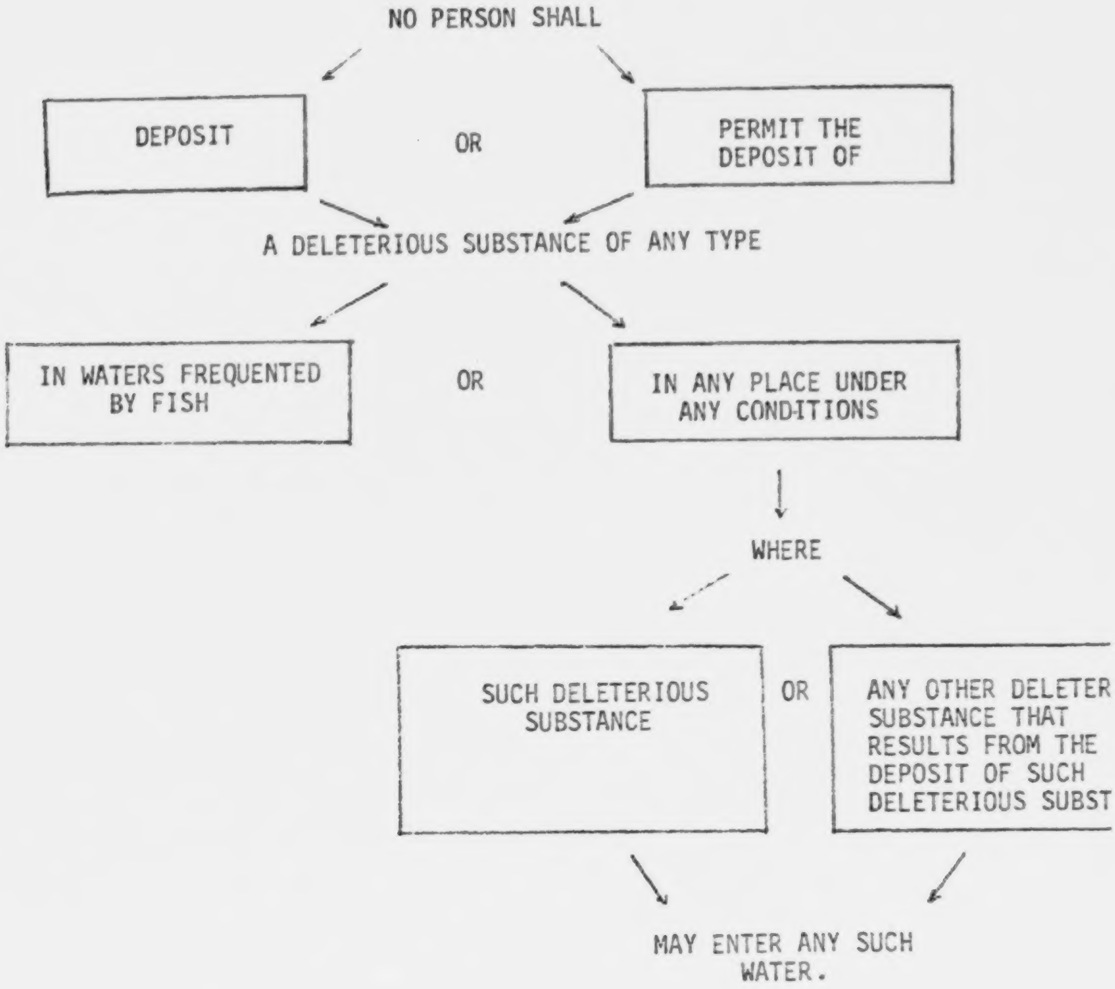
The present Pulp and Paper Effluent Regulations require continuous flow for both the effluent and the control test and 100% survival in the control test for 96 hours. The volume of effluent required for a continuous flow test is considerably greater than required for a static test. The cost of trucking barrels of effluent back to the lab is considerable. The security necessary to ensure continuity of possession with respect to large samples also presents additional problems.

In developing regulatory toxicity tests, the regulation or guideline should prescribe the test in the simplest possible terms. More fish, more replicas, or modifications within the simple terms prescribed may improve the accuracy of the test in particular circumstances. However, unnecessary terms in the regulation will impose additional costs and inconvenience on the regulatory agency with no assurance that the results in court will be any better.

APPENDIX

ANALYSIS OF SUBSECTION 33(2)
OF THE FISHERIES ACT

Subject to subsection (4),


NO PERSON SHALL
DEPOSIT
OR
PERMIT THE DEPOSIT OF
A DELETERIOUS SUBSTANCE OF ANY TYPE
IN WATERS FREQUENTED BY FISH
OR
IN ANY PLACE UNDER ANY CONDITIONS
WHERE
SUCH DELETERIOUS SUBSTANCE
OR
ANY OTHER DELETER SUBSTANCE THAT RESULTS FROM THE DEPOSIT OF SUCH DELETERIOUS SUBST
MAY ENTER ANY SUCH WATER.

ACTUAL WORDING OF SUBSECTION 33(2)

> Subject to subsection (4), no person shall deposit or permit the deposit of a deleterious substance of any type in water frequented by fish or in any place under any conditions where such deleterious substance or any other deleterious substance that results from the deposit of such deleterious substance may enter any such water."

Analysis of Subsection 33(4) of the Fisheries Act

No person contravenes subsection (2)

by depositing or permitting the deposit

in any water or place

(a) of waste or pollutant

of a type,

in a quantity and

under conditions

authorized by regulations
applicable to that water or place

made by the Governor in Council
under any Act other than this Act; or

(b) of a deleterious substance

of a class,

in a quantity or concentration and

under conditions

authorized by or pursuant to regulations
applicable to that water or place or
to any work or undertaking or class thereof,

made by the Governor in Council
under subsection 33(13).

Analysis of Definition of Deleterious Substance in Subsection 33(11) of the Fisheries Ac

"Deleterious substance" means

(a) any substance that,

if added to any water,

would degrade or
alter or
form part of a process of
degradation or
alteration of

the quality of that water

so that it is rendered or is likely to be rendered

deleterious to fish or
fish habitat or
to the use by man of fish
that frequent that water, or

(b) any water that

contains a substance in such quantity or
concentration, or that has been so treated,
processed or changed, by heat or other means,

from a natural state,
that it would

if added to any other water

degrade or
alter or
form part of a process of degradation or
alteration of

the quality of that water

so that it is rendered or is likely to be rendered

deleterious to fish or
fish habitat or
to the use by man of fish
that frequent that water,

and without limiting the generality of the foregoing includes and without limiting the generality of the foregoing includes

(c) any substance or
any substance that is part of a class of substances

prescribed pursuant to paragraph (12)(a),

(d) any water that contains

any substance or
any substance that is part of a class of substances

in a quantity or concentration
that is equal to or in excess of
a quantity or concentration

prescribed in respect of
that substance or class of substances
pursuant to paragraph (12)(b), and

(e) any water that has been subject to

a treatment,
process or
change

prescribed pursuant to paragraph (12)(c).

SCHEDULE D

TEST FOR DETERMINING TOXICITY OF MILL EFFLUENT

1. The sample of mill effluent to be tested shall be maintained at a temperature not exceeding 8°C from the time that it is taken until the time of the test.

2. For the test, two tanks equal in size are to be used.

3. The liquid in one tank shall be a mixture of 65 per cent effluent and 35 per cent water, the latter being taken from the same water supply that is being used to hold fish to be used in the test.

4. The liquid in the other tank shall consist entirely of water taken from the same water supply as that being used to hold fish to be used in the test.

5. While the test is being conducted

(*a*) the liquid in each tank shall be continuously aerated, and

(*b*) the liquid in each tank shall be replaced continuously at a constant rate by fresh water or fresh effluent mixture as the case may be.

6. An equal number of fish that appear to be healthy and that are of a species that frequent the waters into which the mill effluent is being discharged shall be placed in each tank and kept there for a period of 96 hours.

7. If any of the fish in the tank that does not contain effluent fail to survive for the period of 96 hours, the test shall be repeated.

8. If all the fish in the tank that does not contain effluent survive for 96 hours and less than 80% of the fish in the tank that contains effluent survive for ninety-six hours, that

Cornwall*, G.M., and E. Pessah**. 1979. The relationship between 'Best Practicable Technology' and Adequate Environmental Protection. Proc. Fifth Annual Aquatic Toxicity Workshop, Hamilton, Ontario, Nov. 7-9, 1978. Fish.Mar. Serv. Tech. Rep. 862, pp. 46-57.

My presentation today has a rather ominous sounding title, "The Relationship between Best Practicable Technology and Adequate Environmental Protection". An obvious question is: Does such a relationship exist?

- BPT is based on economics and the technology that exists to control pollutants.
- Adequate Environmental Protection is achieved when such things as the living organisms in the aquatic environment can live in "harmony" with the products that are discharged into the receiving environment.

In theory there need not be a relationship, but in practice I am confident that there is.

With this brief statement and leaving an unanswered question for the moment, I would like to describe our water pollution control policy at the federal level. Our policy is not based entirely on the analysis of specific phenomena. It is instead a product of relational judgements about knowledge and ignorance of effects, about social and political values, about technical feasibility, and about industrial and other costs. The growth of knowledge, (something which workshops such as this have contributed to greatly) and the management of that knowledge are essential. The need to perceive often subtle signs of future problems is more critical than ever. Equally important, however, are the management of ignorance -- yes, I said ignorance -- and the application of value judgements.

Discussions on the management of ignorance and some of the grey areas or

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information gaps should see much more exposure at workshops such as this. It is equally easy to criticize decisions based on the extrapolation of few data. It can be argued that there is no such thing as not taking a decision because data gaps exist. Not making a decision in such circumstances is in effect, a decision not to act. When scientists identify gaps, I think there is a need to recommend the manner in which such gaps can best be filled.

A principal purpose of water pollution control is protection. Right away we have a problem - protection from what? The simple answer is "from levels of pollutants which cause adverse effects". But this, as you know, then raises the questions - what levels - what substances? We are into the grey world of risk analysis - where that which is black and white is rare and that which is contentious or controversial is commonplace. The scientific data is never quite complete enough to satisfy critics of a decision. There is often controversy about the interpretation of such data, frequently encouraged by those with a vested interest in casting doubt on the interpretation. Rarely is information presented adequately to the public in the media. This is a lack for which both the media and governments often bear responsibility. In short, the achievement of complete consensus on the wisdom of controlling a given substance to a given level is a most uncommon event. Yet determine risks and act to reduce them, we must.

The prevention and control of pollution in Canada is effected through numerous pieces of federal and provincial legislation. At the federal level, pollution prevention and control provisions of the Fisheries

Act prohibit the deposit of substances deleterious to fish or man's use of fish into waters frequented by fish.

The policy of the Federal Government is, in the first instance, to control industrial pollution at source through national baseline standards in the form of regulations applied at the effluent pipe. More stringent measures can be applied using the provisions of the Fisheries Act as well as using other federal or provincial legislation as required to adequately protect the environment.

There is a particular aspect of the control of pollutants at source which warrants special attention. I refer to possible differences between new (or substantially changed) plants and existing ones. From both an engineering and an economic perspective, it is probable that in many instances new plants would be able more readily to achieve higher effluent quality than existing operations. Does it therefore follow that they should? I believe that the answer is yes. We are in a state of knowledge growth that is best described as dynamic, perhaps even exponential. Generally speaking, that growth has increased rather than reduced our environmental concerns. In Canada, as well as in many other countries working progressively to ensure that damage to the environment is minimized, there is increasing emphasis of concern directed towards those contaminants which result in long standing and potentially drastic changes in the nature of the living environment. The dilemma is that these changes often begin in a very subtle way and are frequently difficult to detect let alone fully understand. It is obvious to all of us, I am sure, that as man's activities and industrial processes become more and more sophisticated, the potential for imposing these contaminants (either as products or as residuals) on the receiving environment will continue to increase. In such circumstances it is only common sense to design new operations to anticipate and protect the future by minimizing to the extent feasible the introduction of pollutants into the environment.

Having discussed some of the platitudes, and by the use of this word I in no way intend to negate the importance that must be placed on the factors I have mentioned, I should now indicate the manner in which 'best practicable technology' is built into the federal effluent requirements developed under the Fisheries Act.

The national baseline regulations and/or guidelines are developed by the Environmental Protection Service. The basic categories are industrial sectors which have been priorized according to probable overall impact on the Canadian environment. For each category, a Task Force involving technical people represents federal and provincial governments and industry. The Task Force examines the technical options available (including process modifications and add-on wastewater treatment units), and recommends a definition of best practicable technology (BPT). This process identifies specific parameters and limits that characterize the industrial waste leaving a well-operated industrial site from the viewpoint of minimum discharge of substances deleterious to fish as defined in the Fisheries Act. Regulations and/or guidelines are developed for each industrial sector based on the recommendations of the government/industry Task Force. To date, regulations and guidelines have been developed for pulp and paper, oil refinery, and chlor-alkali (mercury), base metal mining, meat and poultry, potato, and metal-finishing sectors, are in various stages of development for a number of other industrial sectors.

I need to make a momentary diversion at this point to elaborate on the practicable part of the term BPT. Effective August 1, 1978, the Federal Government instituted a new requirement in the process of establishing regulations of the type we have been developing under the Fisheries Act. A socio-economic impact analysis (SEIA) must be conducted for each proposed regulation which would require industry, nationally, to invest more than ten million dollars in capital in any one year. Similar impact analyses are being required in many other countries.

The new SEIA requirement will involve publishing an intent to regulate along with a statement summarizing the cost-effectiveness of the proposed regulatory controls.

Coming back to the scientific and technical aspects of our national effluent requirements, it would be ideal if science were able to tell the policy maker, with certainty, how much of what substances will have what effect. There are some who argue that no control action should be taken until science can give us specific answers of this kind. I said earlier that one of our principal challenges is to manage ignorance and nowhere can this need be better seen than in our efforts to address risks and act to reduce them. We have to make educated guesses. We have to apply subjective judgements - and I submit to you that those judgements should be coloured by considerable prudence, in short a bias in favour of protection.

The impact of an effluent on the receiving water includes the results from an integrated action of oxygen demand, suspended solids, pH, nutrients, heavy metals and numerous chemical compounds, the presence of many which may be determined, at least in part, by fish bioassay procedures. Most of the information on fish toxicity has been derived from laboratory studies carried out under controlled conditions. What is apparent is that the much more difficult analysis of the impact of pollution on the natural ecosystem has lagged behind. Notwithstanding the significant contribution of many at this meeting, there remains much to do.

There is no doubt that a situation in which we could make use of the resilience and capacity of the environment with confidence, and I stress the word confidence, would be most attractive from several points of view. As I have said, science has not yet brought us to this point. Despite these knowledge gaps we are able to take necessary action.

I have already discussed our point source control approach but I would now like to emphasize the value of toxicity in this approach. In this regard I feel rather like the fellow who carried coal to Newcastle! However, if you will excuse an engineer, and a managerial type at that, discussing a scientific tool like toxicity tests at a seminar attended mainly by biologists who have spent much of their career working in this field, I will put on my hard hat and my safety toe shoes and proceed.

Typically, national effluent limitations are expressed in terms of allowable discharge of a given weight of contaminant per unit of production for two to six chemical parameters along with a toxicity limit requirement. The chemical parameters that are selected and the limits that must be achieved dictate the level of control that must be exercised on the effluent.

In the case of the petroleum refinery effluent controls, for example, the requirements have also been designed to generally encourage water conservation and segregation of non-contaminated from contaminated effluents. The assumption here is that it is more efficient to control pollution in-plant and/or to treat reduced quantities of more concentrated wastes with resultant conservation of dollars, energy and raw materials. At the same time, considerable concern had been expressed by industry that the toxicity limit requirement in fact tended to increase rather than decrease water usage. An operation with reduced water usage would yield a more concentrated, hence more toxic, waste. The problem was recognized and a dilution allowance was introduced for those plants with exceptionally low water rates. The toxicity requirement (at least 50% survival of rainbow trout in a 96-hour toxicity test) was also set in accordance with BPT, and is used as a "catch-all" or perhaps more appropriately as an "integrator" to limit the loss to the environment of deleterious substances that are not specifically regulated. Toxicity control in this context is an important and significant element in the control program.

The alternative to a toxicity test would be a comprehensive chemical monitoring of each effluent. Because of the myriad of chemical species usually found in industrial wastewaters, this is an impractical, if not impossible, task. Even if it were practical, such an approach is incapable of describing the nature of the waste relative to biological systems. While the regulatory toxicity test in use today under the Fisheries Act has limitations, it does provide an integrative measurement taking into acount many of the biologically interactive (i.e. synergistic, antagonistic, additive) qualities of complex industrial wastes.

Of the variety of tests that could be used as a measure of toxicity, the mortality bioassay is regarded as most appropriate for regulatory bioassays because:

1. Mortality is an "all-or-none" response and since the data collected may be used as evidence in a court of law, a test procedure giving a definitive "yes" or "no" answer that requires a minimum of interpretations is most desirable;
2. it is indicative of the level of protection defined by BPT;
3. it is a relatively simple test to perform; the more complex the test, the greater the possibility for error; and
4. it is economical; the test should not be more expensive than necessary to test compliance with regulations.

It is important at this time to restate that this test is designed to test for compliance with a regulation and not to investigate the biological effects of industrial wastes on fish. It is clear that the existing test does not take into account ecologically significant sublethal effects. It is equally clear, however, that the sensitivity of the existing test is well suited to the needs of ensuring the application of BPT. A more sophisticated and sensitive bioassay test would not be any more useful at the moment in curbing pollution through the application of

BPT. Parameters which the toxicity test itself can not address (mercury, PCB's, oil, etc.) are or will be addressed through specific controls to complete the picture.

The overall effectiveness of the water pollution control program, namely the impact of the program and of the toxicity test in protecting or enhancing the aquatic environment, has hardly begun. The relationship of toxicity at the end of the industrial pipe and the state of the environment beyond the pipe will only be understood when the results of long-term trend studies of the aquatic systems affected by effluent regulations are undertaken and analyzed. In the meantime, the argument of control at source versus control by site will continue. We have chosen to control pollution by improving baseline standards and by superimposing more rigorous site-specific standards as required. To accomplish the first level of protection, general effluent quality objectives, limits based on a simple toxicity test and few chemical parameters, can accomplish the task efficiently.

I would like now to briefly cite a couple of examples to illustrate the argument:

1. Not very long ago, the St. Croix River bordering New Brunswick and Maine appeared to be helpless and hopeless as a salmon stream. Bark deposits, suspended solids, and dissolved oxygen problems were of concern. The implementation of requirements consistent with our own BPT and toxicity controls at a large pulp mill in Maine has resulted in much improved water quality. A study was done this year and is

expected to be published soon, which indicates that conditions have improved to the point that salmon can be reintroduced.

2. I have already made mention of the petroleum refinery effluent controls. They were issued in 1973 and address parameters of oil and grease, phenols, sulphide, ammonia, total suspended matter and pH as well as acutely lethal toxicity.

A joint PACE-EPS study was recently completed on the final effluent from a refinery whose normal chemical characteristics were close to but below allowable discharges and which was normally non-lethal to rainbow trout. One of the objectives of the study was "...to determine the concentrations causing sublethal effects for a liquid oil refinery effluent that complied with Canadian government regulations" and with the toxicity guideline.

The report concluded that "Dilution of such an effluent by about 200 times would apparently preclude sublethal effects. Such dilution would probably be attained fairly rapidly at most refinery sites." In the context of ensuring adequate environmental protection, sites having insufficient dilution potential would be studied for negative environmental effects and more stringent requirements imposed as required.

3. A large pulp and paper complex at Kamloops on the Thompson River system was designed and built with BPT and toxicity control in mind. The spectacular Adams River run of sockeye -- and I can attest to the impressiveness of the spawning process on the Adams River having walked

that stretch of the river just this fall -- appears quite able to exist in fine form in spite of the pulp and paper mill discharge which is controlled to the BPT level.

There are, I am certain, many members of this audience who are of the opinion that the application of uniform national standards, including an acute lethality limitation, does not adequately protect the environment, or fish in particular. On the other hand, there may well be many who believe that compliance with effluent controls involves the wasteful expenditure of money. To address these critics, I must say that, to date, we have seen very little evidence that the implementation of national controls does not or will not provide for adequate protection of fish. In addition, the non-lethal objective (as measured by the 96 hour acute lethality test) that we embody in our effluent limits represents,

in our view, an objective that typifies a common decency level that all dischargers of deleterious sustances should be setting their sights upon.

In concluding I will simply state:

1. The ideal is that we so manage the control of water pollution that we will function comfortably within environmental limits while taking full advantage of the resilience and capacity of nature and of the aquatic organisms.
2. The reality is that science has not yet given us the data nor the mechanisms needed to manage the environment in this ideal fashion with confidence.

3. The conclusion is that we must increase our research efforts in order to bring us closer to the ideal.

4. The need is to ensure that in the interval - which may last for some considerable time - we maintain a posture of prudence with a particularly strong bias in favour of protection where environmental effects are expected and where new installations are to be controlled. The tool to meet this need is the techology-based effluent limit, the stringency of which should reflect the bias just identified and other socio-economic considerations.

5. The hope is that such an approach will provide a reasonable level of protection and, properly explained to the public, contribute to improving the credibility of regulatory programs.

6. And finally, I agree that there is not an exact or algebraic relationship between best practicable technology and environmental protection but I personally am confident that the level of environmental protection that has been achieved when BPT has been installed is generally adequate. In those instances where it is judged inadequate, more stringent, site specific requirements can and will be imposed.

Howard*, T.E. 1979. Toxicity standards as modifiers of industrial operations. Proc. Fifth Annual Aquatic Toxicity Workshop, Hamilton, Ontario, Nov. 7-9, 1978. Fish. Mar. Serv. Tech. Rep. 862, pp. 58-62.

ABSTRACT

The various regulatory jurisdictions in Canada apply toxicity standards to industrial effluent discharges. Using examples from the pulp and paper industry, this paper shows how the existence of such standards has required newer mills to install secondary treatment facilities. Older mills have commonly made process changes and substantial in-plant improvements which, in part, have resulted from the need to meet toxicity standards. Further, research is underway to make major changes in the mill process which is largely justified by the improvement in effluent toxicity and BOD_5 that will result if such work is successful.

The paper concludes with a brief general discussion of the function of bioassay testing at industrial plants and for monitoring purposes.

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At the first of these Workshops, I served as "Devil's Advocate" in a paper which suggested that toxicity testing in Canada was disjointed; quality control of fish condition and testing methods were rudimentary and the logistics of effluent sampling and shipment were, in some instances, horrendously complex. Now, five years later, I have been invited to discuss the practical aspects of toxicity testing as it relates to industry. Many of the aforementioned problems remain but, on this occasion, I chose to emphasize the positive aspects of toxicity testing.

The chief virtue of the toxicity standards contained in the various regulations affecting effluent discharges across Canada is their existence. Despite the problems of definition, standardization, interpretation and implementation associated with its development, the bioassay test is the sole biological criterion of effluent quality and, as such, is a far more powerful tool with which to define the probable impact of an industrial waste upon the environment than are more conventional physical and chemical analyses of effluent. To the best of my knowledge, no other country demands that industry satisfies a biological criterion of acceptable effluent quality at the point of discharge of the waste. Using examples from the pulp and paper industry, I hope to show that the need to meet toxicity standards has modified the process operations of this industry, and in some locations, has directly resulted in the installation of external-to-plant treatment systems.

APPLICATION TO THE INDUSTRY

The Classic Case

No example better illustrates the impact of toxicity standards on this industry than that associated with the development of integrated forest complexes including bleached kraft mills on the Fraser River during the mid-1960's. Research by the International Pacific Salmon Fisheries Commission, the then Department of Fisheries of the Federal Government and work on behalf of the companies examined the probable effects of these effluents on fish and sought methods to minimize any adverse effects of the mills on the river. It was rapidly established that, at the dilutions available, there was no likelihood of dissolved oxygen depletion in the receiving waters as a result of dissolved organics in the effluent (measured as BOD_5). On the other hand, it was determined that the high volume of effluent (approximately 40 million gallons per day) was weakly toxic (96-hr LC50, 15-60% v/v).

Thus, before development of the mills was allowed, requirements were set for toxicity. According to these requirements, the mills needed to produce an effluent in which juvenile coho salmon could survive with no mortality for 96-hr in a 65% v/v effluent concentration. This requirement was largely based on the findings of the International Salmon Fisheries Commission (Servizi, Stone and Gordon, 1966) whose report read, in part,

> "Experiments with biological treatment of the waste indicated that, although the initial and chemical strength of the waste varied widely, a reduction of biochemical oxygen demand by about 60%, would render the waste almost non-toxic to fingerling sockeye".

The association of aerobic microbiological treatment (sic "biological treatment") with effluent detoxification was the sole basis on which these mills installed such treatment systems. Nowadays no new mill on this continent or in Scandinavia would be developed without such an external-to-plant treatment system.

In the 13 years, following their initial operation, these mills and all others in the interior of B. C. have installed treatment facilities and have undergone continuing toxicity assessment by provincial and federal regulatory agencies. Recently, as a result of effluent treatment problems, two of these original mills on the Fraser River have combined in a 12-million dollar expenditure to upgrade their initial treatment systems.

Impact on Operating Mills

The need to comply with the various regulations on effluent quality, particularly the toxicity standards, has prompted changes to the process operations of many Canadian pulp and paper mills and has encouraged research into new ways to modify the pulping and bleaching process in order to remove the need for external to-plant treatment. Without going into details of such activities, they can be briefly summarized as follows: In the manufacture of pulp, the sulphite process has lost favor,particularly those sulphite processes which do not allow for the recovery of lignin and degradable materials produced in the initial digestion of the wood ahead of any subsequent bleaching stages. Small sulphite mills in this country, the U.S.A. and most particularly in Scandinavia have closed down while large mills have shifted from calcium base to other cooling chemicals such as magnesium, which allow for the installation of chemical recover systems. Alternatively, as in the case of the Canadian Cellulose mill at Prince Rupert, B. C., the operation has been shifted over to the kraft process.

Older kraft mills have radically upgraded their operation by bringing together the multiple outfalls that characterized such old mills and by installing additional liquor storage capacity and spill systems within the mill. Commonly, in-plant monitoring of BOD_5 and toxicity will be carried out to evaluate the effectiveness of these upgrading programs.

Frequently, the bringing together of the multiple outfalls is the precursor to the installation of a single deep diffuser. It is a practical reality that such programs which upgrade the quality of the effluent produced in-plant and then which dispose of the final effluent through well designed diffuser outfalls achieve substantial rehabilitation of the environment, without the need in all instances for aerobic secondary treatment systems. In British Columbia, this is acknowledged in the regulations by the concept of an initial dilution zone near a mill outfall (for which there are criteria) and with different levels of required treatment and toxicity objectives which relate to mill age and location. In federal regulations there is no such distinction in the toxicity standards which have been established.

New mills and those which are in environmentally sensitive locations have,at present, no practical alternative to the installation of secondary treatment systems. Here, the incentive for in-plant control is economic, bearing on savings of chemicals or energy, and at least in part is designed to smooth out variations in BOD_5 and toxicity of the effluent entering the biobasins.

Studies at our laboratory and elsewhere, have shown that surges in influent quality can greatly disrupt the performance of biobasins and cause the final effluent to fail toxicity tests.

A number of other areas are being actively pursued by the industry to reduce effluent volume and to upgrade effluent quality to the point where external-to-plant treatment may not be required. The most exciting of these is at the Great Lakes Paper Company, Thunder Bay, Ontario, where the "Rapson-Reeve" closed mill system is being tested on a mill scale. Presently, this system has not realized its full potential but, if successful, will mean that the production of pulp can be undertaken with essentially zero effluent discharge. There is no doubt that the option of not needing a waste treatment facility is a strong selling point of this system.

Elsewhere, a range of research and process development studies are underway, all of which are favored by their ability to reduce effluent toxicity and BOD_5. Oxygen delignification which allows further recovery of toxic waste materials and reduces the chlorine demand of mills is a commercial reality. Alternative bleaching systems have been tested at numerous mills; such systems rarely reduce costs to the mill but may reduce toxicity for the same cost. Water reuse and recycle options are being actively pursued at all mills. Further, it is now common practice to test proposed new process additives for their toxicity to fish before accepting them for mill use.

IMPLICATIONS FOR THE FUTURE

Hopefully, the preceding has convinced you that there has been a real benefit in environmental protection as a result of the existence of toxicity standards in this country and that the practical application of bioassay testing has enabled us to realize these benefits. However, in concluding this presentation, I would like to return to my opening remarks concerning the nature and function of the acute toxicity test for industrial discharges, the need for standardization of test procedures and to offer a word of caution concerning the uncritical application of bioassay numbers for regulation. Gaddum (1944) described death as the "ultimate asymptomatic response" and this is an appropriate caution. Lethal bioassays, particularly those with complex industrial wastes, are not likely to give biologists directly relevant data with which to predict the impact of a waste upon specific ecosystems. Such conclusions can only be drawn "after applying the full armamentarium of our trade", including sublethal testing and detailed ecological assessment. Rather, lethal bioassays measure whether a waste is extremely toxic to the degree that direct lethality would occur in the environment or, more normally, whether in-plant or external-to-plant treatment of the waste has been undertaken to an extent which approaches the best, or at least an appropriate level of technology.

Blind faith in the values of an LC50 of an industrial effluent is equally improper; particularly where an industry has husbanded resources by internal reuse and recycle, reducing effluent volume but perhaps increasing the toxicity of the effluent in terms of concentration. Overall, this will normally result in a reduced discharge of toxicants. Biologists must learn to think more in terms of toxicity standards based on a loading basis, as embodied in the "toxic unit" concept.

At this and similar gatherings, we debate the protocol to undertake the optimal bioassay test. Certainly, this is both necessary and desirable, although one could be forgiven for thinking there is too slow progress towards the utilization of truly standardized test fish. It is incongruous that industry is asked to make costly decisions when the "analytical chemicals"--in this case the fish--are too often not standardized in terms of age, condition, tolerance to a reference toxicant and, ideally, genotype. Be that as it may, in the search for the optional bioassay procedure, which is so necessary for legal testing or research investigations, it is important not to lose sight of the intrinsic role, value and limitations of the routine test data which are most likely to be used as the basis for dialogue between regulatory agencies and industry. For routine testing, methods are needed which follow the basic format and break none of the rules of toxicology. At the same time, the tests should be sufficiently simple that it can, and will, be used with enough frequency to fulfill a useful monitoring function. An adversary situation in court is a special case. But generally, however, it is more useful to present enough data to show a pattern of performance, perhaps illustrating the adverse effects of periodic spills of strong liquor or the effects of seasonal variation in treatment plant performance. In my experience, industry personnel can be convinced of the need to take remedial steps in such cases. In the text of this presentation examples are given of the way that toxicity data are being used to encourage research into process modifications or,where necessary, to support the purchase of appropriate aerobic treatment systems. In either case, bioassays have proven their worth in protecting our aquatic environment.

Metikosh[*], S. 1979. Industrial application of the toxic unit concept. Proc. Fifth Annual Aquatic Toxicity Workshop, Hamilton, Ontario, Nov. 7-9, 1978. Fish. Mar. Serv. Tech. Rep. 862, pp. 63-79.

INTRODUCTION

Federal regulations are currently being established to govern the acute toxicity of industrial effluents. Because these effluents often consist of combined intermediate process streams contributing unknown quantities of toxicity, more than one type of treatment technology may be required to remove specific toxic components. The toxicity of final effluents could be considerably reduced if major toxic process streams are identified and treated separately.

The toxicity of process streams can be determined by 96-hr bioassays, however, the resultant LC50's are independent of flow and do not indicate the effects of individual process streams on the toxicity of the combined effluent. There is a possibility that this difficulty could be overcome by relating flow and toxicity to provide a quantitative measurement of the toxicity of each contributing stream.

Esvelt et al (1973) incorporated this concept in a study that determined the toxicity discharged into San Francisco Bay from municipal and industrial sources. Their study defined the toxicity of each effluent as the toxicity concentration (TC) which was represented by the equation:

$$TC = \frac{100}{\text{96-hr LC50 (\%)}} \qquad (1)$$

The units for TC were toxic units (TU) as identified by Sprague and Ramsay (1965). The study demonstrated that the toxic unit contribution of each effluent to the receiving water could be determined by the product of the toxicity concentration and the ratio of the effluent flow to the combined flow.

A joint study by the Environmental Protection Service, Ontario Ministry of the Environment, Shell Canada Ltd., and Imperial Oil Limited was conducted to determine the feasibility of using this method to assess both in-plant toxicity and toxicity reduction capabilities of wastewater treatment systems of petroleum refineries.

The toxicity contributed by each process stream to its corresponding branch of the main oily water sewer is expressed in toxic units and defined by the equation:

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$$TU_p = TC_p \times \frac{Q_p}{Q_{ows}} \qquad (2)$$

where TU_p = the toxic units contributed to the oily water sewer by the process stream

TC_p = toxic concentration of the process stream $\frac{100}{96\text{-hr LC50 (\%)}}$

Q_p = flow of the process stream

Q_{ows} = flow of the oily water sewer

If the toxicities contributed by the process stream are additive, the sum of the toxic units from each process stream should equal the number of toxic units in the oily water sewer and a toxicity balance would be established. Such a balance would permit a quantitative identification of each process streams' contribution to the toxicity of the oily water sewer, and the subsequent isolation of highly toxic streams which may require specialized waste treatment.

MATERIALS AND METHODS

The experimental program was carried out on the oily water sewer system and wastewater treatment facilities of the Shell Canada Ltd. refinery in Corunna, Ontario. The oily water sewer system (Figure 1) consisted of one main stream fed by two tributaries from the two process areas. Effluent from the main oily water sewer was treated by the oily water separator, air floation unit and activated sludge process before being discharged.

Sample locations for the toxicity tests were grouped into the following three sets:

Set 1 - Stations 11A, 11B, 11, 13, 15 and 16, the branch sewers, main oily water sewer and the treatment process effluents;

Set 2 - Stations 1, 2, 3, 4 and 11A, the No. 1 crude unit, the three process effluents shown in Figure 1 and the oily water sewer;

Set 3 - Stations 6, 7, 8, 9, 10 and 11B, the No. 2 crude unit, the four process effluents shown in Figure 1 and the oily water sewer.

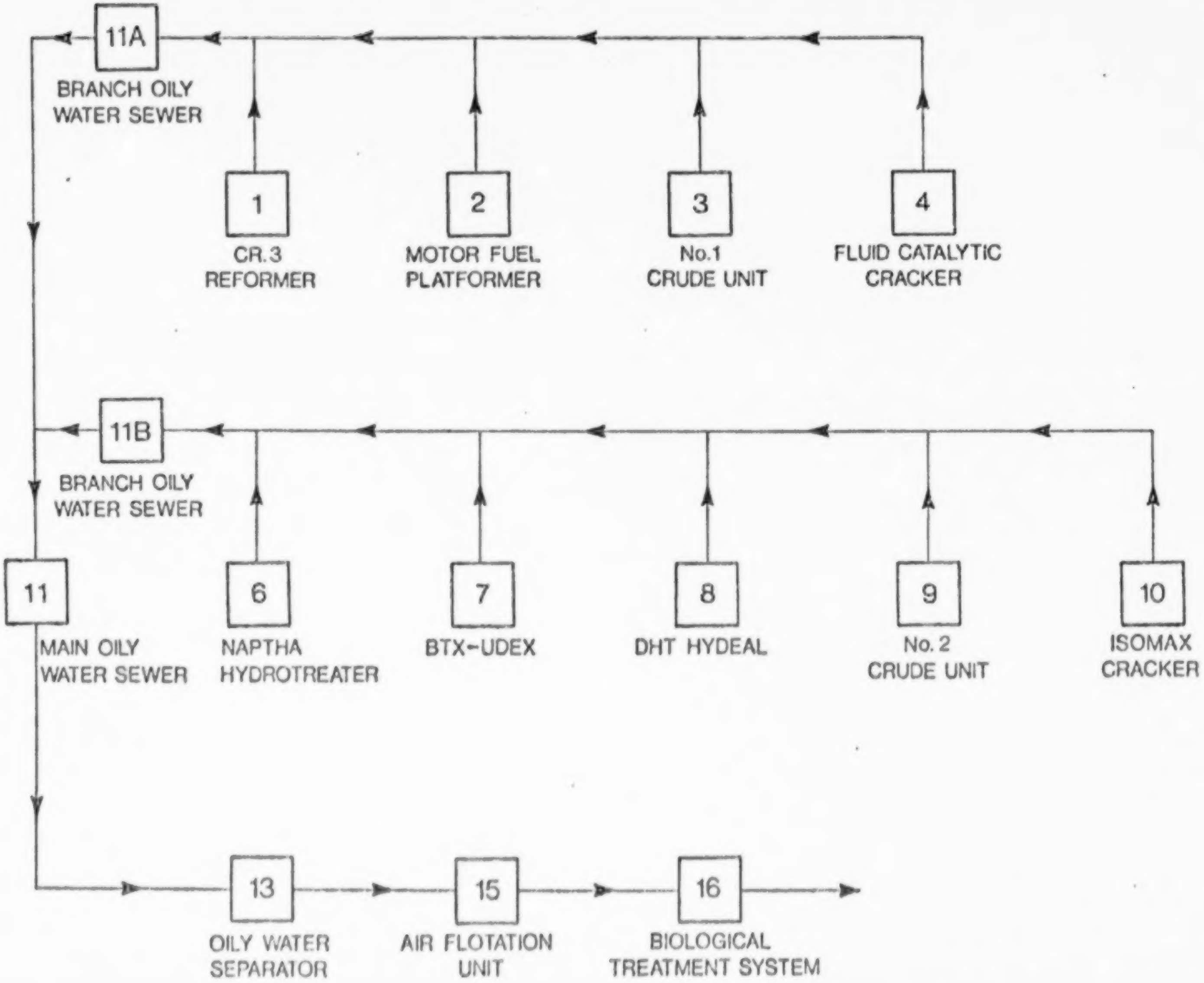
Flow measurements for the process streams were calculated from material balances. The flows of 11A, 11B and 11 were determined by a dye injection technique using Rhodamine B. Flows within the treatment system were metered by a Parshall Flume.

Bioassays were conducted in a mobile bioassay laboratory equipped with fish holding tanks, facilities for temperature control and dechlorination of water, the dilution equipment for six continuous flow bioassays, two of which were replicated. A second trailer equipped with four 5 400-litre and two 2 700-litre refrigerated fiberglass tanks was used for effluent storage. Samples were stored at 5 ± 1°C for the duration of the test.

The dilution apparatus used was a Mount-Brungs diluter capable of delivering five concentrations and a control. Each diluter was calibrated to divide a sample to proportions of 100, 50, 25, 12.5, and 6.25 percent by volume. Maximum concentrations delivered by the diluter were 100, 50, 25, 15, 10, 5, 1.0 percent by volume. The diluters cycled once every 5 min ± 15 sec to provide a 90 percent molecular replacement every eight hours in the test container. Twenty-litre polyethylene buckets fitted with polyethylene liners were used as test vessels. The average loading density in the test containers was 7.3 L/g/d. The test containers were immersed in a water bath to maintain a constant temperature of 15°C.

A disease free stock of rainbow trout (Salmo gairdneri Richardson) were used in the bioassay. Fish were held in 700-litre circular fiberglass tanks supplied with a continuous flow of 4.0 L/min of dechlorinated municipal water. The test fish ranged from 1.0-2.5 g in weight and 4.0-6.0 cm in length. Fish were fed a daily ration of dry pellet food. No feeding occurred 24 hours prior to the bioassay.

The experimental program operated on a seven day schedule. The first day of the study was devoted to taking an 8-hr composite sample from all the streams in the set. The following day an 18-hr preliminary continuous flow bioassay was conducted to determine the maximum concentration to be used in the 96-hr bioassay. The diluters were recalibrated to deliver this pre-determined maximum concentration and the bioassay started on the third day. Ten fish were randomly distributed into each of the 48 test containers. Test vessels were observed at elapsed times of 0.25, 0.5, 1, 2 and 4 hours and every two hours thereafter for the duration of the 96-hr test. The number of fish alive, number dead, and number experiencing equilibrium loss was recorded for each observation time. Dissolved oxygen and pH were measured prior to introducing the fish, and every four hours


11A
BRANCH OILY WATER SEWER
1
CR.3 REFORMER
2
MOTOR FUEL PLATFORMER
3
No.1 CRUDE UNIT
4
FLUID CATALYTIC CRACKER
11B
BRANCH OILY WATER SEWER
11
MAIN OILY WATER SEWER
6
NAPTHA HYDROTREATER
7
BTX-UDEX
8
DHT HYDEAL
9
No. 2 CRUDE UNIT
10
ISOMAX CRACKER
13
OILY WATER SEPARATOR
15
AIR FLOTATION UNIT
16
BIOLOGICAL TREATMENT SYSTEM

for the length of the test. Dead fish were removed from the test containers at the time mortalities were observed and weight and fork lengths were recorded. Routine observations were made on the remaining three days of the test. Bioassays were completed on the seventh day. A portion of the sample collected from streams 11, 11A, and 11B, September 4th was stored for seven days at 5° ± 1°C before testing.

LC50's with confidence limits were calculated for exposure times of 24, 48, and 96-hrs using the method described by Litchfield (1949). In cases where no partial responses were observed the LC50 was estimated on log-probability paper. The limits of the LC50 were defined by the concentration at which 100 percent and 0 percent mortality occurred.

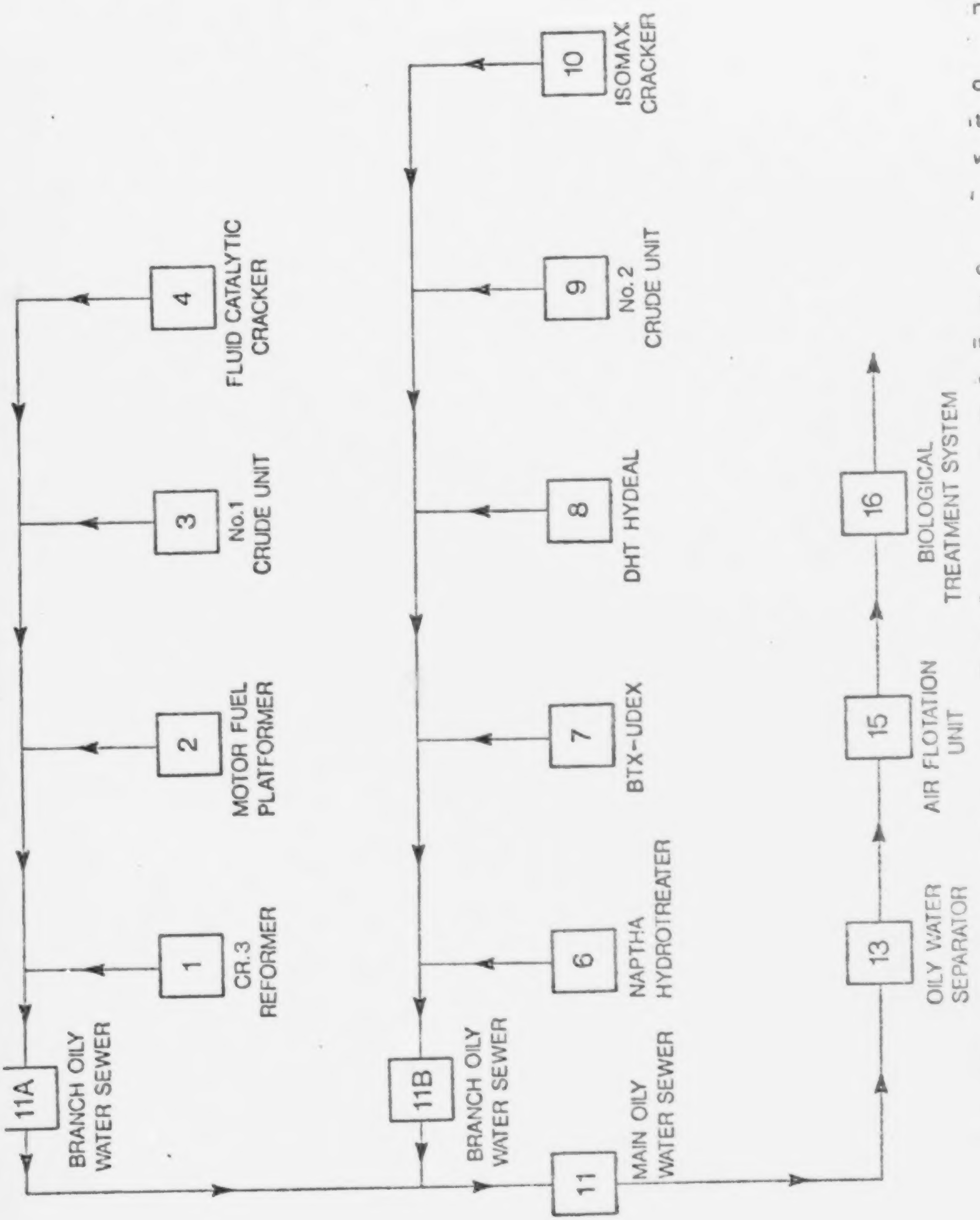
The number of toxic units contributed by each process stream to the oily water sewer was determined by multiplying the toxicity concentration of the process stream by the fraction of the total flow it contributed to the oily water sewer. Toxic units in the process stream were calculated using equation 2. A toxicity balance existed when the sum of the toxic units from all of the process streams equalled the observed number of toxic units in the oily water sewer.

RESULTS

Twenty-four, 48 and 96-hr LC50's were determined for the treatment system, Set 1, and the process effluents, Sets 2 and 3 (Table 1). Analysis of variance indicated no significant difference ($p \leq 0.05$) between 24, 48, and 96-hr bioassays for all streams or between the LC50's from replicated bioassays. Ninety-six hr LC50's could not be determined for the oily water sewers 11A and 11B on July 16 because of reduced sample volumes. In this case the 96-hr LC50's was replaced by 48-hr LC50's in the toxicity balance calculation.

The effects of seven days storage on the toxicity of samples from Streams 11, 11A and 11B are reported in Table 2 and indicate that approximately 50 percent of the toxicity could be lost.

LC50's were converted to toxic units using data from Table 1 and the corresponding flow data reported in Table 3. The toxic unit contribution of individual streams and the toxic unit calculations are presented in Figure 2, 3 and 4.


11A
BRANCH OILY WATER SEWER
1
CR.3 REFORMER
2
MOTOR FUEL PLATFORMER
3
No.1 CRUDE UNIT
4
FLUID CATALYTIC CRACKER
11B
BRANCH OILY WATER SEWER
6
NAPTHA HYDROTREATER
7
BTX-UDEX
8
DHT HYDEAL
9
No.2 CRUDE UNIT
10
ISOMAX CRACKER
11
MAIN OILY WATER SEWER
13
OILY WATER SEPARATOR
15
AIR FLOTATION UNIT
16
BIOLOGICAL TREATMENT SYSTEM

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oily water
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11.2 TU
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TABLE I. SUMMARY OF BIOASSAY RESULTS

Date	Stream	24-hr LC50	Upper	Lower	48-hr LC50	Upper	Lower	96-hr LC 50	Upper	Lower
July 16 Set 1	11	3.6	4.28	3.02	3.05	3.96	2.34	3.05	3.96	2.34
	11A	1.8	2.14	1.5	1.8	2.14	1.5	**1.8	-	-
	11B	2.7	*3.25	1.87	2.7	*3.25	1.87	**2.7	-	-
	11BR	1.8	2.1	1.53	1.8	2.1	1.53	**1.8	-	-
	13	20.0	23.0	17.9	17.0	22.0	13.0	15.2	*25.0	12.5
	15	2.38	3.04	2.22	2.38	3.04	2.22	2.38	3.04	2.22
	16	26.0	*50.0	25.0	27.0	32.0	22.0	17.5	*25.0	12.5
Aug. 7 Set 1	11	7.9	*12.5	5.0	7.9	*12.5	5.0	7.0	*12.5	5.0
	11A	3.5	* 5.0	2.5	3.5	* 5.0	2.5	3.5	* 5.0	2.5
	11AR	3.5	* 5.0	2.5	3.5	* 5.0	2.5	3.5	* 5.0	2.5
	11B	2.05	2.7	1.55	1.85	2.5	1.4	1.85	2.5	1.4
	11BR	1.75	* 2.5	1.25	1.75	* 2.5	1.25	1.75	* 2.5	1.25
	13	13.5	16.0	11.3	11.8	14.0	9.4	11.5	*12.5	5.25
	15	11.5	*12.5	6.25	12.0	21.6	6.6	12.0	21.6	6.6
	16	>100	-	-	>100	-	-	>100	-	-
Sept 4 Set 1	11	3.5	* 5.0	2.5	3.5	* 5.0	2.5	3.5	* 5.0	2.5
	11A	4.5	5.6	3.7	3.5	* 5.0	2.5	3.5	* 5.0	2.5
	11AR	3.5	* 5.0	2.5	3.5	* 5.0	2.5	3.5	* 5.0	2.5
	11B	4.5	5.4	3.7	4.5	5.4	3.7	3.5	* 5.0	2.5
	11BR	3.5	* 5.0	2.5	3.5	* 5.0	2.5	3.5	* 5.0	2.5
	13	NOT SAMPLED								
	15	12.0	14.0	10.3	11.9	14.2	9.9	11.5	*12.5	6.25
	16	>100	-	-	98.0	100	74.0	70.0	75.0	64.0
Aug. 15 Set 2	1	8.8	*12.5	6.25	8.8	*12.5	6.25	8.8	*12.5	6.25
	2	48.0	51.0	45.0	46.0	50.0	37.5	30.0	33.0	26.0
	3	1.45	* 2.5	1.25	1.45	* 2.5	1.25	1.45	* 2.5	1.25
	3R	1.35	1.59	1.13	1.3	1.5	1.1	1.7	2.89	1.0
	4	14.5	*25.0	12.5	11.0	16.0	7.5	7.4	11.0	4.9
	11A	17.5	*25.0	12.5	17.5	*25.0	12.5	17.5	*25.0	12.5
	11AR	17.5	*25.0	12.5	17.5	*25.0	12.5	17.5	*25.0	12.5
Aug. 27 Set 3	6	>100	-	-	>100	-	-	>100	-	-
	7	>25.0	-	-	>25.0	-	-	22.0	26.0	19.0
	8	7.0	8.2	5.9	7.0	8.2	5.9	6.4	7.2	5.6
	9	0.25	0.31	-	0.25	0.31	-	0.25	0.31	-
	9R	0.40	0.54	0.29	0.33	0.38	0.27	0.31	-	-
	10	60.0	*75.0	50.0	54.0	58.0	49.0	54.0	58.0	49.0
	11B	8.9	*12.5	6.25	8.9	*12.5	6.25	8.9	*12.5	6.25
	11BR	10.5	*12.5	6.25	10.5	*12.5	6.25	10.5	*12.5	6.25

* LC50 Estimated on log-probability paper--limits: conc. 100% mortality; conc. 0% mortality

** 96-hr LC50 Estimated from 24-hr and 48-hr LC50

R - Replicated

TABLE 2. EFFECTS OF STORAGE ON THE 96-HR LC50

Stream	Before Storage	Stored for 7 days at 4°C
11	3.5 (5.0-2.5)	5.0 (6.5-4.7)
11A	3.5 (5.0-2.5)	7.3 (8.6-6.0)
11AR*	3.5 (5.0-2.5)	7.8 (12.5-6.25)
11B	3.5 (5.0-2.5)	7.0 (10.0-5.0)
11BR*	3.5 (5.0-2.5)	5.7 (10.0-2.5)

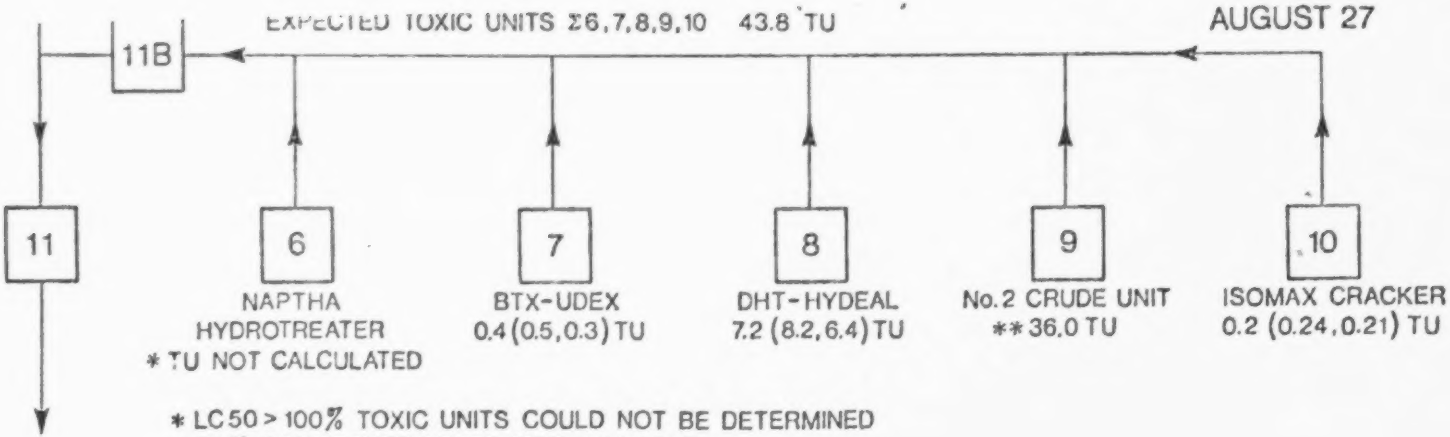
* Replicated samples

TABLE 3. FLOW FRACTIONS USED IN TOXICITY BALANCE CALCULATIONS

Date	Set	Stream	Flow Fraction
July 16	1	11A	0.36
		11B	0.64
		11	1.0
August 7	1	11A	0.40
		11B	0.60
		11	1.0
September 4	1	11A	0.34
		11B	0.66
		11	1.0
August 15	2	1	0.01
		2	0.34
		3	0.26
		4	0.31
		11A	1.0
August 27	3	6	0.24
		7	0.09
		8	0.46
		9	0.09
		10	0.12
		11B	1.0

ON THE 96-HR LC50

Storage	Stored for 7 days at 4°C
0-2.5)	5.0 (6.5-4.7)
0-2.5)	7.3 (8.6-6.0)
0-2.5)	7.8 (12.5-6.25)
0-2.5)	7.0 (10.0-5.0)
0-2.5)	5.7 (10.0-2.5)


EXPECTED TOXIC UNITS Σ6,7,8,9,10 43.8 TU
AUGUST 27
11B
11
6
NAPTHA HYDROTREATER
* TU NOT CALCULATED
7
BTX-UDEX
0.4 (0.5,0.3) TU
8
DHT-HYDEAL
7.2 (8.2,6.4) TU
9
No.2 CRUDE UNIT
** 36.0 TU
10
ISOMAX CRACKER
0.2 (0.24,0.21) TU

* LC50 > 100% TOXIC UNITS COULD NOT BE DETERMINED
** 95% CONFIDENCE LIMITS NOT AVAILABLE

FIGURE 2. TOXICITY BALANCE CALCULATIONS FOR THE PROCESS STREAMS

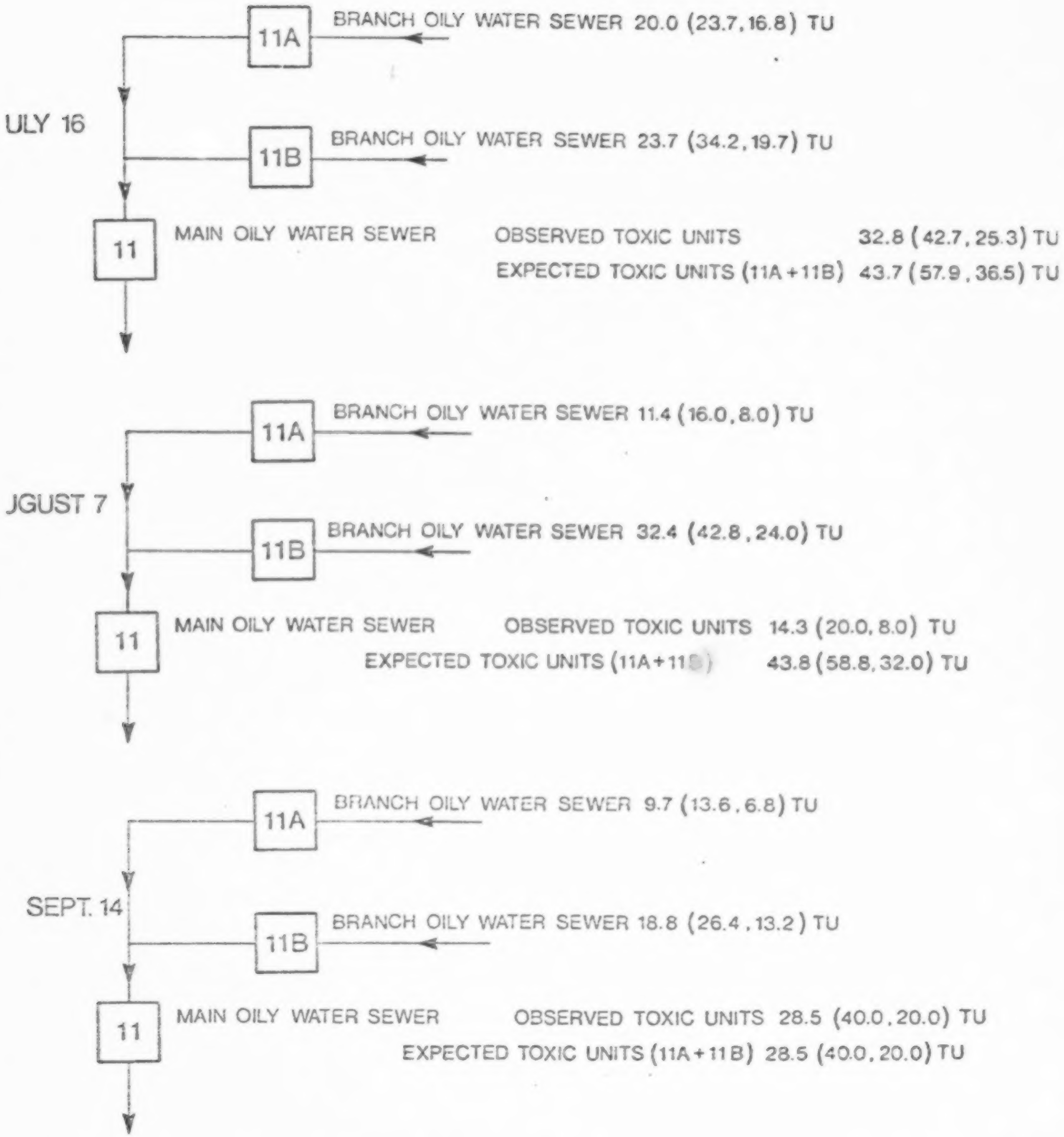

11A
BRANCH OILY WATER SEWER 20.0 (23.7, 16.8) TU
ULY 16
11B
BRANCH OILY WATER SEWER 23.7 (34.2, 19.7) TU
11
MAIN OILY WATER SEWER
OBSERVED TOXIC UNITS 32.8 (42.7, 25.3) TU
EXPECTED TOXIC UNITS (11A+11B) 43.7 (57.9, 36.5) TU
11A
BRANCH OILY WATER SEWER 11.4 (16.0, 8.0) TU
JGUST 7
11B
BRANCH OILY WATER SEWER 32.4 (42.8, 24.0) TU
11
MAIN OILY WATER SEWER
OBSERVED TOXIC UNITS 14.3 (20.0, 8.0) TU
EXPECTED TOXIC UNITS (11A+11[illegible]) 43.8 (58.8, 32.0) TU
11A
BRANCH OILY WATER SEWER 9.7 (13.6, 6.8) TU
SEPT. 14
11B
BRANCH OILY WATER SEWER 18.8 (26.4, 13.2) TU
11
MAIN OILY WATER SEWER
OBSERVED TOXIC UNITS 28.5 (40.0, 20.0) TU
EXPECTED TOXIC UNITS (11A+11B) 28.5 (40.0, 20.0) TU

FIGURE 3. TOXICITY BALANCE CALCULATIONS FOR THE OILY WATER SEWER SYSTEM

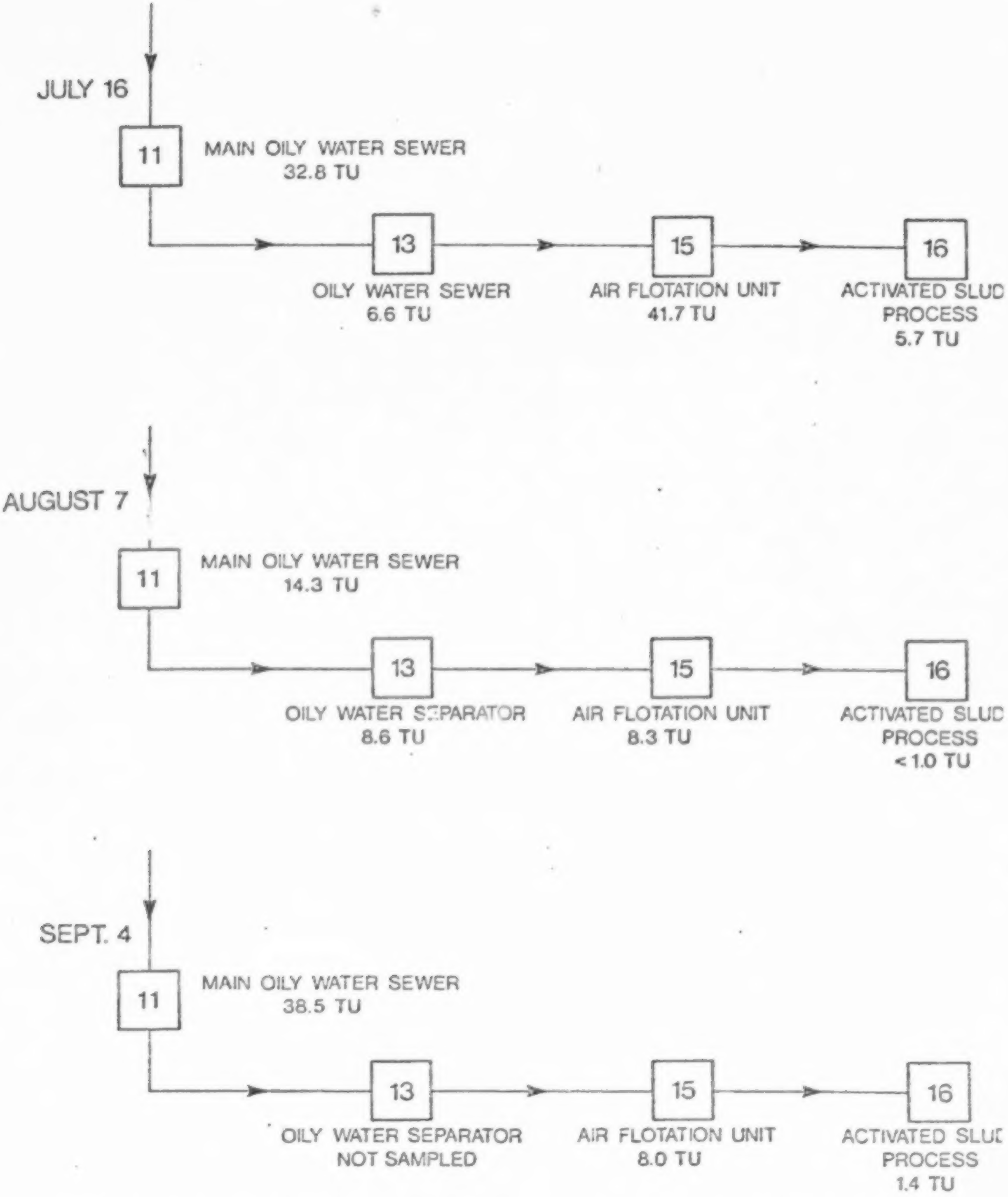

JULY 16
11
MAIN OILY WATER SEWER
32.8 TU
13
OILY WATER SEWER
6.6 TU
15
AIR FLOTATION UNIT
41.7 TU
16
ACTIVATED SLUD
PROCESS
5.7 TU
AUGUST 7
11
MAIN OILY WATER SEWER
14.3 TU
13
OILY WATER SEPARATOR
8.6 TU
15
AIR FLOTATION UNIT
8.3 TU
16
ACTIVATED SLUD
PROCESS
< 1.0 TU
SEPT. 4
11
MAIN OILY WATER SEWER
38.5 TU
13
OILY WATER SEPARATOR
NOT SAMPLED
15
AIR FLOTATION UNIT
8.0 TU
16
ACTIVATED SLUI
PROCESS
1.4 TU

FIGURE 4. TOXICITY REMOVAL BY THE TREATMENT PROCESS

DISCUSSION

A toxicity balance did not exist between the process stream (1 to 5) and Stream 11A. Similarly, a balance did not exist between the process streams (6 to 10) and Stream 11B. In Sets 2 and 3, the main sources of toxicity were the crude units, Streams 3 and 9, respectively. The sum of toxic units for the process streams excluding the contribution from the crude units was within the confidence limits of the observed toxic units for the oily water sewers. The reduced number of toxic units in 11A and 11B indicated that the toxicity initially measured in the crude unit effluent was no longer present in the oily water sewer. In addition, results indicated that synergism did not occur between any of the streams sampled.

Toxicity balance may be unattainable when process effluents contain volatile and highly interacting materials. It is speculated that a significant proportion of toxicants may be lost through stripping of volatiles, or antagonism resulting from interaction with other substances.

A toxicity balance was more readily attained between the oily water sewers (11A and 11B) and the combined effluent (11). The oily water sewers may have been more stable than the process streams due to thorough mixing and a possible loss of volatiles between the process streams and the oily water sewer. Inability to achieve a balance (August 3), was due in part to the unusually high toxicity of Stream 11B.

Effluent from Stream 11 was treated by the oily water separator followed by air flotation and biological treatment. More than 70 percent of the incoming toxicity was removed in the oily water separator or the air flotation unit or both. There was insufficient data to determine which of the two systems was responsible for the largest portion of toxicity removal. Effluent from the air flotation unit was more toxic than the influent on the July 16 sample. Since all samples were collected simultaneously without regard for lag time between treatment processes, it is possible that this toxicity reflected the variability of the wastewater being treated by the air flotation unit.

The biological treatment system consistently reduced the toxic units from the air flotation unit by 85 percent. Toxicity removal remained constant regardless of fluctuations in influent toxicity. However, effluent from the activated sludge process was toxic on several occasions. During bioassays in which the biologically treated effluent was non-acutely toxic, fish in the 100 percent concentration lost equilibrium and changed colour, indicating severe stress after exposure. Such effects may be due to refractory toxicants not readily amenable to biological treatment or due to the activated sludge process not operating at optimum conditions.

Although treated effluents do not reach incipient lethal level within 96-hrs, the incipient lethal level for process effluents was established within the first 24-hrs. All of the process streams reached the incipient LC50 within the first 24-hrs and showed little or no difference between the 24, 48 and 96-hr LC50's. Either the process streams contained substances which exerted toxicity early in the bioassay or toxicity was lost during the first 24-hrs in the storage tank. Storage for seven days indicated that approximately 50 percent of the toxicity was lost through storage. Loss of toxicity could have resulted from a loss of volatile toxicants or from chemical interactions during the storage period. The different rates at which process streams and treated effluents affected rainbow trout suggests two types of toxic action and consequently, two different types of toxic components. The first would be an extremely toxic volatile fraction which could be rapidly removed by the oily water separator and/or the air flotation unit, and the second, a persistent non-volatile fraction which could not always be removed by biological treatment.

Failure to achieve a toxicity balance between the process streams and the oily water sewer does not prove that this concept cannot be used as a tool for solving industrial waste treatment problems. Results from this study indicate that useful information can still be obtained even though a toxicity balance cannot be established.

The two crude units (Streams 3 and 9) were the most toxic process streams in the refinery. On the basis of bioassay and flow data, it is postulated that a significant reduction in the oily water sewer toxicity could be achieved if these streams were isolated for specialized treatment. However, the inability to establish a toxicity balance indicated that the toxic units contributed by the crude units were not present in the oily water sewer and that separate treatment would not significantly reduce the toxicity of the oily water sewer.

FACTORS TO BE CONSIDERED IN APPLYING THE TOXIC UNIT CONCEPT

This study was a practical application of a theoretical concept and some of the operational difficulties encountered can provide useful information for planning future experimental programs where the toxic unit concept is used.

Flow measurement is a critical parameter for toxicity balances. Mechanical flow measurement is the most reliable means of obtaining flow data. However, it is often impossible or impractical to use this method due to the inaccessibility of some industrial process streams. Flow measurements using a tracer provide a suitable alternative to mechanical methods. In this study a tracer was used to determine the flow proportions of Streams 11A and 11B to 11. A known concentration of the tracer was injected into the oily water sewer and the concentration was measured at a point downstream. The flow was estimated from the dilution ratio and the tracer addition rate. The choice of the tracer used is important for obtaining accurate results. The tracer should be non-acutely lethal to allow flow measurement on the day of bioassay sample collection. The characteristics of the wastewater should also be taken into account when selecting the tracer. The absorption of the tracer by the wastewater may introduce errors in the concentration measured downstream. In addition some of the wastewater components may interfere with the instrumentation or the analytical procedures required to measure tracer concentration.

Tracer studies could not be conducted on the process streams because downstream sampling points were usually not available. Flow proportions for Sets 2 and 3 were determined from material balances calculated from analytical data obtained for the process streams and the oily water sewers. The chemical nature of the process streams and the extreme fluctuations in the effluent characteristics throughout the sampling period made the selection of suitable analytical parameters from which material balances could be calculated extremely difficult. Consequently, this method of determining flow proportions proved to be the least reliable.

In studies where relative flows are to be determined from material balances, careful consideration should be given to the nature of the effluent, the selection of parameters to be used in the balances and the difficulties associated with chemical analysis of complex effluents.

The development of a toxicity balance requires the accurate assessment of the toxicity contributed by each process stream. Wide fluctuations in the toxicity of industrial process streams can occur over a period of eight hours. Composite samples represent the average wastewater characteristics during sample collection but peaks of toxicity are often masked by the effects of dilution. Consequently, the actual toxicity of the stream cannot be determined. It is possible that a large number of grab samples collected at various times would give a better estimate of the process stream toxicity.

Although process effluents reached the incipient lethal level within 24 hours, evidence of residual toxicity in the final effluents indicated that the incipient lethal level for treated effluent could not be established with a 96-hr bioassay. In future studies the length of the bioassay should be determined by the time required to reach the incipient lethal level. For the process streams, the amount of information obtained could be increased significantly by replacing 96-hr bioassays with 24-hr tests. Conversely, information concerning the toxicity of treated effluents could be lost by terminating bioassays after exposure for 96-hrs.

Fluctuation in the toxicity of the process streams and the oily water sewer reduced the accuracy of the 96-hr LC50. Although the 18-hr continuous flow, preliminary bioassay indicated a maximum concentration for the 96-hr test, there was no indication of the lowest concentration. Consequently, a wide range of concentrations was required to ensure that the 96-hr LC50 was bracketed by the highest and lowest concentrations. Since the Mount-Brungs diluter was capable of delivering only five concentrations it was necessary to use a 50 percent dilution factor to cover the required range. Such a large dilution factor contributed error to the LC50 by reducing the possibility of attaining partial responses and thus preventing the calculation of 95 percent confidence limits. Where fluctuations in toxicity exist, the accuracy of the LC50 can be increased by maintaining a wide range of concentrations and increasing the number of intermediate concentrations. A ten-concentration diluter with a 25 percent dilution factor would provide a range of concentrations from 100 to 7 percent, thereby reducing the possible error in the LC50 by 50 percent.

CONCLUSIONS

This study determined the feasibility of using the additive nature of toxic units in assessing the toxic contributions of refinery process streams to the combined effluent. Although toxic units had proven to be additive when used to describe simple mixtures of several toxicants they were not additive when this concept was applied to actual industrial effluents. The inability to achieve a toxicity balance resulted from extreme fluctuations in the toxicity of the process streams and the combined effluent; the presence of volatiles; possible antagonistic chemical reactions; and operational difficulties associated with sampling procedures, flow measurements and chemical analysis of complex wastes.

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ABSTRACT ONLY

The new institutional arrangements established in the late 60s and early 70s for dealing with environmental problems (e.g., Departments of the Environment at all levels of government) have permitted enormous progress in modifying and in some cases reversing pollution trends. A further step is now necessary: transformation to an ecosystem/biospheric approach. The advantages of a "man-in" ecosystem approach are: (1) it deals with a unitary whole (a system, rather than a part of a system); (2) it provides a basis for improved human motivation ("man-in," rather than "man-out:"); (3) it avoids counter-productive issues such as jobs versus environment; (4) it connects us to a hard limit (carrying capacity of the biosphere); and (5) it links us to our origins and capabilities for future change (evolution; origin of life and man).

Two examples are used to illustrate the above: road salt; human motivation with respect to future generations and life.

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ABSTRACT

A discussion is presented of two problems pertinent to the use of water quality objectives. The first concerns a lack of specification of the element of time in the statement of specific water quality objectives. Difficulty results in interpreting the significance of situations in which the desirable level of water quality is not met during some times of the year. The second problem concerns the selection of a water quality data base for comparison with the objectives. A special type of water quality monitoring program is required for this purpose.

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Introduction

As a valuable natural resource, fresh water serves a number of functions. Probably the most important to man is the provision of water for public supply, for use in industrial processes and in agriculture. Rivers also have a commercial or an amenity value as natural habitats supporting fish and other aquatic organisms and plant life, and are used for human recreation and transport. Each of the uses requires water of a largely definable quality, which is frequently threatened by the other major uses of rivers in their function as receptors for industrial and domestic wastes and their natural but frequently man-assisted function of land drainage. In terms of the quality needed, the most demanding uses are usually for potable supply and fish and aquatic life. If requirements for these are satisfied, with some exceptions the quality is sufficient for other purposes.[1]

Successful river management involves the maintenance of a balance between the requirements of competing uses, with pollution control playing an obvious role in regulating the major sources which degrade water quality. The debate on the relative merits of different policies or administrative systems for the control of water pollution has been going on for many years in most jurisdictions, and the lack of clearcut superiority in any one method is illustrated by the fact that there are several practices in different parts of the world.

World approaches to the establishment of water pollution control fall generally into two categories based on the location at which standards are applied (to the source or to the receiving water) and a third approach based on discharge fees. Effluent and in-stream standards can be applied in a variety of ways depending on the degree of uniformity desired and the way in which the standards are set. Uniform effluent standards require that the quantity of a pollutant discharged be less than a fixed level. This kind of standard may be developed nationally without regard to the nature of the source, as used to be the case in Britain, or it may be developed as in Canada by setting minimum national standards to apply across individual industrial sectors. The in-stream counterpart of uniform effluent standards is a system of water quality standards developed to cover a broad geographic area by mandating the quality of the receiving water. Broad-scale in-stream standards were in use federally in the U.S. until 1972. Lower down on the geographic scale are local effluent standards, such as those used in Britain, comprising a system of effluent controls developed individually for each discharge so as to provide a certain degree of protection to the receiving environment. Their in-stream counterpart involves the development of water quality standards for all or parts of a watershed, a practice followed by several of the American states. Zero discharge standards, the kind of uniform effluent standard which is the goal of U.S. regulation development, find their counterpart in the environmental philosophy of non-degradation. Lastly, the philosophically distinct system of user fees, practiced by countries such as Germany, requires that polluters pay a tax for each unit of pollutant discharged. This system relies on economic penalties to produce a clean environment.

The Canadian approach to water pollution control embodies two elements from among those just mentioned:

- "an overall national program that will limit pollutants at their source for specific sectors and that will apply uniformly across Canada;
- a site specific approach involving more stringent source controls necessary to meet specific water quality objectives."[2]

The development of specific water quality objectives to support the Canadian regulatory approach of effluent controls began in the Great Lakes region and has subsequently spread across the country. In this context, however, it is worth noting that objectives have been developed for only a small proportion of the rivers and lakes in Canada; a site specific basis for the development of source controls is thus frequently lacking.

In the following discussion, the distinction between water quality objectives and water quality standards should be remembered. The former represent a goal of water use management, while the latter are regulatory standards. The Government of Canada has jurisdiction in the area of water quality primarily through the influence of this quality on fish and other aquatic life and through the control of contaminants hazardous to the environment or to human health.

Derivation of water quality objectives

Some knowledge of the derivation and use of water quality objectives is necessary before the problems inherent in their formulation can be understood. Such an understanding should make some of the desirable characteristics of objectives more apparent and lead, in turn, to the formulation of a different sort of objective.

The problems just referred to arise when water quality data sets are compared to specific water quality objectives. This type of objective can be distinguished from the more general water quality objectives in the following way:

- water quality objective (WQO) - a qualitative statement of general intent with respect to the use of a body of water and the level of water quality required to sustain that use.

- specific water quality objective (SWQO) - a quantitative description of the level of water quality, for a single parameter, which is necessary or desirable to sustain a certain use.

The numerical limits which are set in Canada for individual substances in the Great Lakes, for example, are thus classed as SWQOs. A statement that the water quality in the Saint John River should support fishing and swimming would be an example of a general WQO.

A second distinction that needs to be made is between specific water quality objectives and water quality criteria. This distinction can be illustrated by considering the way in which SWQOs are derived. The starting point in the formulation of an SWQO to protect aquatic life is a statement of scientific fact - a criterion. On the basis of available evidence, a criterion is synthesized which typically gives the highest level of a substance in water which is consistent with producing no measurable biological effect. The foundation for the criterion is experimental evidence. Based on it, an SWQO is derived to meet the goal expressed in the general water quality objective. An SWQO is derived from the criterion to protect one of the uses of water. For this reason, it always reflects social or political values as well as scientific fact and may also reflect one or more elements of technical subjectivity.

SWQOs incorporate an element of subjectivity for several reasons. Value judgements are involved in determining the uses incorporated into the general WQO, so that a resulting SWQO reflects first of all a decision about what uses are desirable. These uses may anticipate future riparian development and in addition may be quite selective. For example, the WQO that a certain segment of river should support the passage of salmonids means that an SWQO might be less stringent than it would have been if the WQO had included the protection of spawning or nursery areas.

An SWQO may also be adjusted to take ambient water quality into account. For example, an SWQO for copper should obviously reflect the facts that copper toxicity can be related to hardness (as well as a number of other things), and that different waters have different hardnesses. The way in which the ambient hardness is selected and a subsequent adjustment of the copper criterion takes place may reflect a good deal of subjective thinking about the definition of background water quality.

Consideration of their end use makes it of questionable value to set SWQOs at levels lower than those which occur in an "uncontaminated" stretch of water within a river basin. While it is desirable from the management point of view to recognize that certain use restrictions may be inevitable, it is impractical to specify an objective that is unattainable due to a natural input of contaminants. Similarly, an SWQO should not be derived from a criterion founded on the no-effect level for trout-perch if there are no trout-perch living or planned in the ecosystem under consideration. The SWQOs may thus reflect a species list as well as "background" chemical water quality.

Because they have to be measurable, SWQOs may also reflect the limits of the currently available technology for routine sampling and analysis. The criteria for pesticides, especially, tend to be adjusted for analytical constraints when SWQOs are being established.

While a criterion may be altered in other ways to arrive at an SWQO, the foregoing discussion should be adequate to demonstrate that there is frequently a justifiable difference between the criteria provided as the result of scientific research and the numbers which appear in a set of specific water quality objectives. Their adjustment does not make the SWQOs any less valid for their intended purpose, but it does mean that the numbers themselves may represent something other than the distillation of an exact science.

Use of SWQOs

As we have seen, the federal approach to water pollution control is to set effluent standards, either on a uniform or local basis. In Canada uniform effluent standards set for an industrial sector are the rule. More rigourous local effluent standards may be set because of sensitive ecological conditions or because of the demands of a comprehensive water quality management program. Here, SWQOs may play an important role in determining the level of effluent quality which must be achieved.

The second major use of SWQOs is as the basis for comparison with existing water quality. Such a comparison might serve to indicate to people concerned with the management of water users that a use conflict or use degradation was occurring. Employed in conjunction with trend analysis of water quality, an SWQO might also indicate that a potential problem was imminent.

As a third important use, comparison of existing water quality with a set of SWQOs could also indicate the success or failure of a program of water pollution control. As effluent standards are only a means to an end, so compliance with effluent standards measures only the efficiency with which the targets of a program of water pollution control are met. The yardstick for the effectiveness of a pollution control program is graduated in increments of environmental quality. SWQOs should mark the division between degradation and enhancement.

Although the desirability of these uses is evident, the purposes for which SWQOs are formulated are frequently not achieved. Two significant problems arose when attempts were made to compare extensive water quality data bases for two rivers in the Maritimes with their respective sets of SWQOs. The case more important to the subject of formulation of SWQOs occurred for the international portion of the Saint John River, New Brunswick. In the case of the Shubenacadie River, Nova Scotia, the major difficulty encountered was related to a sampling bias in the water quality data base.

The problem with SWQO formulation

The first problem with SWQO formulation that arises when a set of SWQOs is compared against a set of water quality data is fundamental. Its cause is the absence of any specification of time in the statement of the typical SWQO. Its origins can be linked to three areas:

- The foundation (criterion) on which an SWQO is based.
- The significance of an SWQO to the use it was developed to protect.
- The duration and frequency of water sampling.

As a general characteristic, SWQOs for the preservation of aquatic life, wildlife and public water supplies are based on criteria which relate effects to a level of exposure. In the case of aquatic life, these criteria are usually assembled from the results of bioassays in which organisms were exposed to constant levels of toxicant for periods of time ranging from days to months.

The constant conditions of the bioassays contrast with the variability of the environment. Almost all water quality data sets are made up of the analytical results obtained from grab samples, which are assumed to represent environmental conditions at a single point in time and space. Within such a data set, the frequency with which a substance exceeds any value within its range at one location can usually be determined by statistical analysis, although the accuracy with which such an analysis depicts the real situation depends greatly on factors such as the size of the data set and the seasonal distribution of water quality sampling.

With few exceptions, SWQOs in Canada are specified as single numbers. The exceptions involve parameters such as temperature or pH, which are usually expressed as ranges. Comparison of an SWQO with a set of data representing water quality at one location over a period of time thus produces one of three outcomes:

(a) all of the data points exceed the SWQO value (in the sense of reflecting a worse water quality),

(b) all of the data points are less than the SWQO value (in the same sense), or

(c) some of the data points lie on either side of the SWQO value.

The significance of outcomes (a) and (b) to an evaluation of whether or not the SWQO is being met is obvious, but the significance of situation (c) during such an evaluation is far from clear.

At first sight, in the case where some of the data are on each side of the limit, it would be tempting to state as the outcome of the evaluation that the SWQO was not being met with some frequency which could be determined by statistical analysis. Such an approach would be correct in the narrow sense, but it would be an administrative simplicity unjustified by the purpose of an SWQO in Canada.

Remembering that the set of SWQOs for the range of substances being considered comprises a more general WQO whose goal is use protection, situation (c) really becomes a question of whether or not use degradation or impairment would occur assuming that the particular data set actually represented the distribution of levels at one point over a period of time. This is obviously not a simple question to answer.

For example, the SWQO for zinc to protect aquatic life in the international section of the Saint John River Basin has been set at a maximum of 0.075 mg/l.[3] At one location in the river a cumulative frequency plot shows that 80% of the data set is less than 0.075 mg/l, and 90% of the data is less than 0.15 mg/l. The maximum is 0.22 mg/l. Examining the justification for the SWQO, it is found that the level was set at 0.1 of the 96 hour LC50 to a salmonid at a representative water hardness. Is it possible that a population of the same salmonid species could tolerate the zinc levels and frequencies just described? Such a question, which is typical of those which arose during a comparison of the SWQOs for the international Saint John River, cannot be answered without a considerable amount of further work. Frequently, an answer would require either laboratory work or a literature search comparable to that made at the time the criterion for zinc was established.

The consequences of evaluating outcome (c) in a strict statistical way, by calling all points above the SWQO "violations" or "exceedences" of the objective, should be examined. Because of the typically lognormal distribution of environmental quality data,[4] the first consequence is that in practical terms one is saying that the water quality must normally be very much better than that specified by the SWQO if the objectives are to be met all of the time. Because the original criteria frequently incorporate a safety factor, accepting what one might call the single point exceedence approach can be seen to be conservative. The error, or additional safety factor, would frequently be an order of magnitude or more.

The second consequence is that innocuous fluctuations in environmental quality could be characterized as problems. Such a situation might be expected to lead to a third consequence, in which resources were spent needlessly, perhaps in a program of water pollution control, to resolve the perceived problem.

The final consequence I wish to mention is that the environmental protection process is partially deprived of an important tool for evaluating the effectiveness of clean-up and prevention programs. The deprivation is partial only to the extent to which outcome (c) is encountered but is, nevertheless, a significant limitation on the usefulness of the SWQOs.

Alternative formulation for SWQOs

As outlined above, the problem with the way SWQOs are formulated is basically the inadequate amount of information contained in a single number. While it might be argued that nothing short of a complete criterion review would contain enough information to compare an SWQO against a water quality data base, an operationally oriented SWQO is most desirable. The type of information needed to make water quality comparisons more useful is the amount of time an organism can tolerate a whole range of levels before a given effect is measurable.

Time versus concentration curves are produced during most criteria-oriented bioassays by virtue of their utility in ED50 formulation. Most bioassays in which a sublethal or a lethal effect is measured include observations about the time at which some proportion of the organisms are affected at each of a series of concentrations. This information is usually lost in the transformation of the bioassay results into a criterion, and yet it would be most useful in a comparison of water quality with an SWQO.

It is suggested that an SWQO should be specified as a plot of time versus concentration, with a time scale ranging from acute mortality at high concentrations at one end, to chronic sublethal effects and eventually the "no-effect" level, at the other. Many of the situations in which parts of a water quality data set lay on either side of an SWQO could thus be resolved by comparing the frequency with which undesirable values were encountered with the type of effect and the duration of exposure needed to produce it. Although this technique would obviously be unsuitable for evaluating the significance of the levels of a toxicant whose effects were irreversible, it is clear that the way in which SWQOs are formulated deserves a re-evaluation.

Water Quality Monitoring for SWQOs

A second type of problem was encountered during a comparison of the historical water quality data for the Shubenacadie River with a draft set of SWQOs. The purpose of the comparison was to identify locations of "background" quality and locations where the objectives were not met. The data base was quite large, containing over 52,000 data points covering a total of 165 stations. The initial comparisons and statistics were done on a computer.

The results of the computer comparison were sufficiently at variance with subjective perception of the river basin to cause a closer examination of the data base. It was found that data collected in response to known environmental quality problems and data collected for surveys over short time periods constituted a large part of the data record. A lack of sampling during non-problem periods and the absence of much seasonal coverage rendered most of the data of little value for defining ambient quality. A decision was made that the data base was too biased to use in comparisons against the SWQOs.

A discussion of the details of water quality monitoring is beyond the scope of this paper. It is relevant, however, to outline the problem because the Canada-US Subcommittee on Water Quality in the Saint John River came to the same conclusion as the Shubenacadie River Working Group: that the requirements for monitoring to provide a basis for comparison with the SWQOs needed to be carefully thought out, and that a monitoring program tailored to this specific purpose was required.

Conclusions

Experience in the comparison of existing water quality with specific water quality objectives has led to the identification of two serious problems:

(1) SWQOs are currently not formulated in such a way that managers concerned with water quality can easily use them as a tool in the evaluation of the effectiveness of pollution control programs and the identification of environmental problems;

(2) Monitoring for comparison with SWQOs requires a specialized sampling program. Even large existing data bases may not be adequate for this purpose.

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ABSTRACT

The federal government is committed to the restoration and protection of water quality and the enhancement of aquatic ecosystems. One of the ways this will be undertaken is through the development of water quality objectives.

The Water Quality Branch of Environment Canada is formulating guidelines to assist water managers across Canada in assessing the water quality necessary to protect all water uses in specific bodies of water.

The scientific criteria necessary for formulating the guidelines should include results from acute and sublethal studies on a variety of organisms, the chemistry of pollutants in water, sediment and biota and the means to apply findings of laboratory studies to the field situation. Difficulties encountered in the interpretation of data, the use of application factors and the incorporation of data into the guidelines are discussed.

The behaviour of pollutants under different environmental conditions should be known because a concentration of a pollutant under one set of conditions will be toxic but have relatively little effect under other conditions. The contributions needed from research scientists is emphasized.

Key words: Canada, Federal Government, policy, water quality, criteria, objectives, pollution, research needs.

Résumé

Le gouvernement fédéral est engagé à hausser et à maintenir la qualité des eaux et à mettre en valeur des écosystèmes aquatiques. La mise au point d'objectifs de qualité des eaux sera un des moyens utilisés pour arriver à cette fin.

La Direction de la Qualité des Eaux d'Environnement Canada est à formuler les lignes directrices qui aideront les gestionnaires des eaux à travers le Canada à évaluer la qualité des eaux nécessaire pour protéger tout emploi des eaux dans des masses d'eaux déterminées.

Les critères scientifiques requis pour la formulation de ces directives devraient inclure les résultats provenant d'études d'effets aigus et sous-létaux chez un grand nombre d'organismes, la chimie des substances polluantes dans les eaux, les sédiments, la faune et la flore et les moyens d'appliquer les résultats des études en laboratoires aux cas sur le champ. Les discussions portent sur les difficultés encourues dans l'interprétation des données, l'utilisation de facteurs d'application et l'incorporation des données aux directives.

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Le comportement des agents pollutants sous diverses conditions environnementales devrait être connu puisque la concentration d'une substance polluante pourra être toxique pour un ensemble de conditions mais n'avoir que peu d'effet sous d'autres conditions. On souligne la nécessité d'obtenir des contributions des chercheurs scientifiques.

Introduction

The federal government has published a pamphlet entitled "A Vital Resource" which outlines the federal policy on inland waters. Policy No. 3 states that "The federal government is committed to the restoration and protection of water quality and the enhancement of aquatic ecosystems through the development of water quality objectives to protect water uses; the application of national effluent regulations and guidelines to control pollution discharges at source; and the control of nutrients and chemical substances which can become dispersed in the environment".

A small group of scientists in the Water Quality Branch of the Inland Waters Directorate in Ottawa has been given the task of writing guidelines to be used in the formulation of water quality objectives. This entails gathering information from existing guidelines, the Canadian Drinking Water Standards, the National Research Council criteria documents and published research. Personal contact is also made with people in research laboratories. Results from Canadian research are taken into consideration as much as possible but they are not always available.

This paper will touch on the types of water use which have to be taken into consideration, the formulation of guidelines for these uses, and the contribution that research scientists can make in this undertaking.

Protection of water quality for specific uses

Each water use, for example, aquatic life support, potable water supply and recreation, have different requirements for water quality, and managers and regulatory agencies have to use judgement in deciding on what this quality should be. The purpose of the water quality objectives guidelines is to assist them in this. Water managers also have to consider that where there is more than one use, the requirements for the most sensitive use must prevail. These decisions need reliable scientific facts on which to base specific limits for pollutants acceptable for water use.

Three terms: criteria, objective and standard should be defined as there are differences in word usage between various groups of people.

Criteria represent the scientific data and existing conditions upon which a judgement is based for the recommended objective to support and protect the water uses.

An objective is a designated concentration of a constituent based on criteria which, when not exceeded, should protect an organism, a community or a prescribed water use or uses with an adequate degree of safety.

A standard is a legally described concentration of a constituent established under statutory authority.

Aquatic environment - a whole unit

In considering the effect and control of pollutants, the area of water in question should be looked on as a complete unit. The chemical composition of water is affected by the types of geological formation and soils surrounding it and beneath it, the organisms are dependent on the type of water they inhabit and they also interact with each other.

Socio-economic factors and the technical ability to monitor for pollutants on a routine basis must also be taken into account.

Initiation of the federal program

The lack of reliable information on possible toxic and other detrimental effects of contaminants has often been felt especially in international negotiations, and thus a program was begun by the federal government to produce national use-guidelines for acceptable levels of contaminants to be used across Canada. The national guidelines are not seen as blanket levels to be used regardless of local water conditions, but to be used as the scientific basis for local objectives, taking into consideration the local chemical and physical composition of the water, flora and fauna and water uses. The effects of different water qualities and other factors on toxicity are discussed in the guidelines if they are known. These guidelines apply only to freshwater as the chemical composition of sea and estuarine waters could alter the behaviour of pollutants. The physiology of marine aquatic life also differs from that of freshwater forms.

Formulation of guidelines

The guidelines have been titled "Water Quality Use Objectives" and the mechanism for developing them is illustrated in Figure 1.

Research information gained from government, university and industry and existing water quality data from government laboratories is assessed by the Ottawa water quality objectives group and draft guidelines are written. These go to specialists in various fields such as health, agriculture and fisheries for critical review and comment. After a final assessment, the guidelines are then redrafted and tabled before the federal government interdepartmental committee on water (ICW), to ensure that all interdisciplinary requirements are met. The guidelines then serve as background working documents for developing, in cooperation with provincial authorities, water quality objectives in regional and local areas across Canada.

The use of the guidelines is illustrated in Figure 2. The items considered are the present and future use of the local water, taking into consideration socio-economic factors including public input, the existing quality of the water and technologies available for controlling effluent quality. Federal-provincial working groups are established to develop and recommend water quality objectives for specific bodies of water, taking the above into consideration and using the guidelines "Water Quality Use Objectives". The objectives are officially adopted after review and approval by both federal and provincial governments.

Specific areas in which the federal government has responsibility for developing water quality objectives in cooperation with other jurisdictions, are areas of "significant national interest" which include boundary and international waters, interprovincial waters, the Territories, waters in Indian

Reserves, national parks and cases where federal jurisdictions such as fisheries and navigation are the major concerns.

Types of criteria to be examined in formulating guidelines

Water quality objectives for aquatic life can only be established from critical reviews of data obtained from acute and sublethal tests designed to look at the many aspects of pollutants on aquatic organisms. These aspects include life cycles, behaviour and field observations on fish, invertebrates and plant life. There is a great deal of uncertainty in decision-making. For example: are the data correct; is the interpretation correct; is the statistical treatment sound; what will the future consequences be of stating that certain levels are acceptable? Only tentative objectives can be proposed, even for substances where a considerable amount of research has been done.

The following list contains some of the information which is needed when assessing pollutant toxicity:

1. The distribution of the pollutant between water and sediment.
2. Will adsorbed pollutants be released from sediments and reenter the aquatic ecosystem?
3. The toxic form of the pollutant; is it present under the condition of the experiment and under natural conditions?
4. Complexes, ligands.
5. Mixtures of pollutants, interactions such as antagonism or synergism.
6. pH affecting solubility, availability of metallic salts.
7. Toxicity of degradation products.
8. Toxicity of metabolites.
9. Bioaccumulation, biomagnification.
10. Whether results are from static or flow-through experiments.
11. Were concentrations of pollutants measured or calculated?
12. Age of the organism.
13. Part or complete life cycle studies.
14. Exposure time.
15. Chronic studies to include one concentration which has no discernable effect.

16. Are physiological changes which have been caused by pollutants of significance to the survival of the organism?

17. That the stress being measured is one caused by the pollutant and not by experimental manipulation.

18. Information for a number of groups of organisms.

19. The feasibility of applying laboratory results to the field situation.

The behaviour of pollutants under different environmental conditions should be known because a concentration of a pollutant under one set of conditions will be toxic but will have relatively little effect under other conditions.

It would be of great assistance if authors could include their thoughts on what the effects would be in a natural system as authors are much closer to the organism being studied. These thoughts could be included in the discussion and would be of great help to those who produce the guidelines.

The objectives for aquatic organisms are usually the most stringent and quite often are at least one order of magnitude lower than those for human consumption. It is, therefore, important that this information is available. Organisms abundant in Canadian waters representative of the major Phyla should be used in research designed to study the effects of pollutants on the aquatic ecosystem. Coarse and sports fish have been shown to be quite different in their tolerance to some pollutants and in areas where the most sensitive fish do not occur, maybe a higher concentration of pollutant could be tolerated by the resident community of aquatic organisms.

Application factors

One way of overcoming the scarcity of data, is the use of an application factor. This is defined by Warren (1971) as the decimal fraction by which one would multiply the LC_{50} of species for different pollutants in order to estimate the concentrations of pollutants that would be harmless to these species in nature. The use of application factors itself also poses the problem of what the factor should be, a fifth, a hundredth of the lethal level? Can an application factor derived from studies with one species be used for another species? Application factors have been quite widely discussed by scientists and currently the following are usually applied by the Ottawa water quality objectives group, though they are not used as a rule of thumb if additional information is available.

When pollutants are known to persist or to biomagnify through the food web an application factor of 0.01 is applied. Where the pollutants have been determined to be non-persistent or non-bioaccumulative in the aquatic environment, but where only acute data are available or significant chronic effects have been found, an application factor of 0.05 is used. When the lowest level in a chronic test is judged to be not very serious, an application factor of 0.2 is applied to the 96 h LC_{50} test results. Some scientists may think that these are not of the right order of magnitude.

Existing guidelines

A number of guidelines have already been drafted by the Ottawa group and these include mercury, zinc, copper, cadmium, and some other metals, and work is beginning on the organics.

Some water quality objectives guidelines have already been proposed for a few areas of Canada, for example, the Yukon and Northwest Territories, Shubenacadie-Stewiacke Rivers and the St. John River Basin. These cover all major water uses, the physical characteristics, heavy metals and ions such as chloride and nitrate. The guidelines were drafted by committees of provincial and federal scientists from a variety of disciplines such as forestry, agriculture, fisheries, and health and welfare. The guidelines will be used to formulate water quality objectives for specific water management areas taking into account the uses to which the water will be put, the natural type of water in each area, and also keeping in mind downstream use beyond the jurisdiction of management.

Summary

The production of water quality objectives guidelines is still in its infancy, but it has begun and will provide some of the necessary information to help water managers across Canada preserve rivers and lakes for the well being of Canadians and the environment.

Consideration of the effects of pollutants on the aquatic environment means looking at the whole picture and not part of it. Many criteria are needed and these include the results from acute and sublethal studies on a variety of organisms, the chemistry of the pollutants in water, sediment and biota and the means to apply findings of laboratory studies to the field situation.

A start has been made on reviewing the literature and producing guidelines which are then made available for review by scientists engaged in specific research. Their recommendations are taken into account in the final documents. Besides the help of the reviewers, initial assistance is needed in the form of research which has been carried out and presented in such a way that the results can be used as criteria for incorporation in the guidelines.

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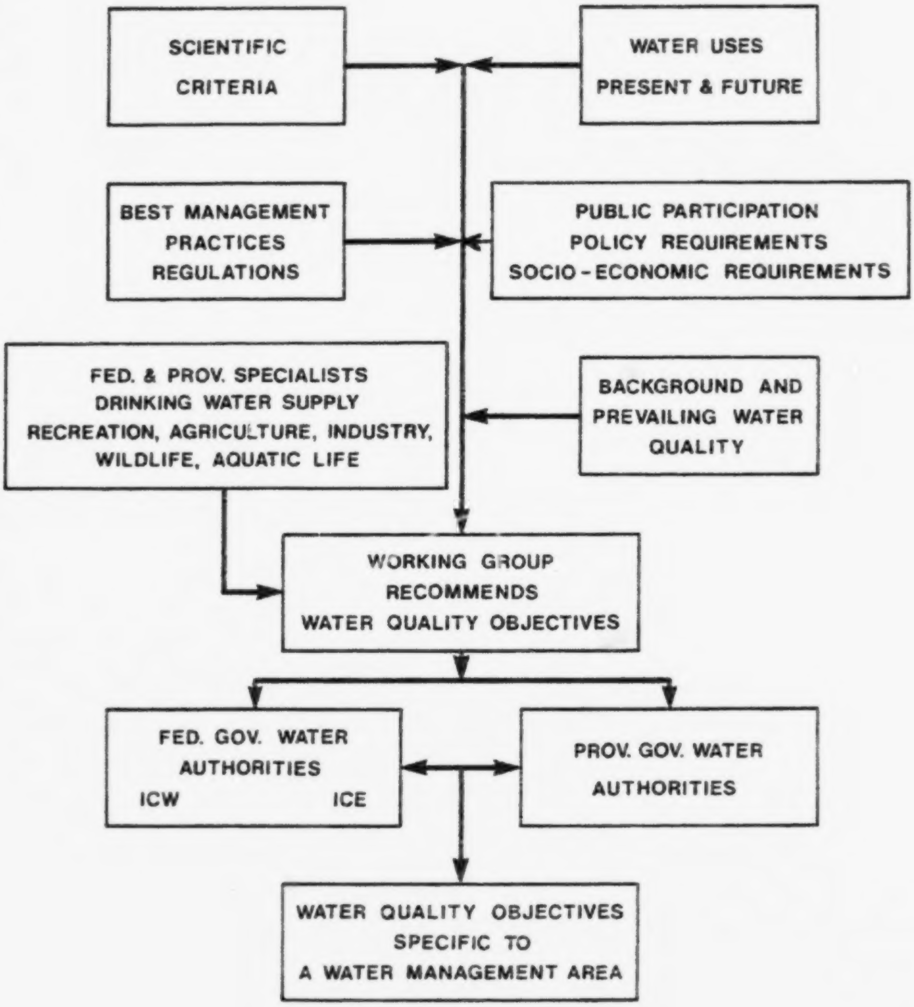
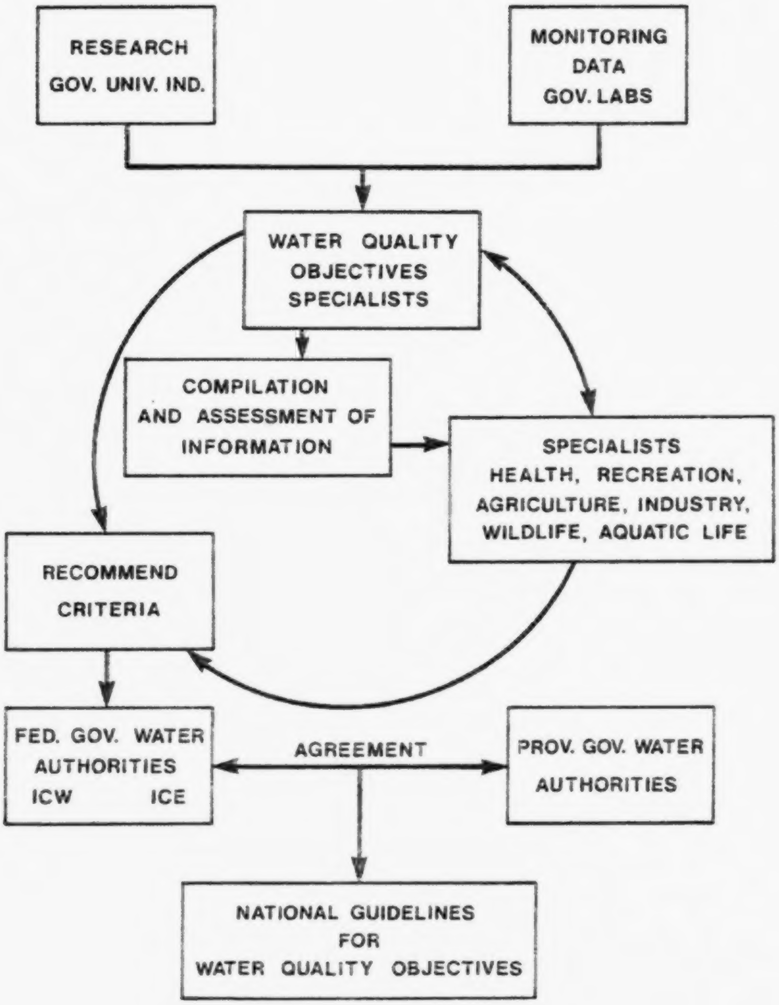

SCIENTIFIC CRITERIA
WATER USES PRESENT & FUTURE
BEST MANAGEMENT PRACTICES REGULATIONS
PUBLIC PARTICIPATION POLICY REQUIREMENTS SOCIO-ECONOMIC REQUIREMENTS
FED. & PROV. SPECIALISTS DRINKING WATER SUPPLY RECREATION, AGRICULTURE, INDUSTRY, WILDLIFE, AQUATIC LIFE
BACKGROUND AND PREVAILING WATER QUALITY
WORKING GROUP RECOMMENDS WATER QUALITY OBJECTIVES
FED. GOV. WATER AUTHORITIES
ICW
ICE
PROV. GOV. WATER AUTHORITIES
WATER QUALITY OBJECTIVES SPECIFIC TO A WATER MANAGEMENT AREA

SCHEME FOR DEVELOPING WATER QUALITY OBJECTIVES
SPECIFIC TO A DEFINED WATER MANAGEMENT AREA
(INTERPROVINCIAL OR INTERNATIONAL WATERS)


RESEARCH
GOV. UNIV. IND.
MONITORING
DATA
GOV. LABS
WATER QUALITY
OBJECTIVES
SPECIALISTS
COMPILATION
AND ASSESSMENT OF
INFORMATION
SPECIALISTS
HEALTH, RECREATION,
AGRICULTURE, INDUSTRY,
WILDLIFE, AQUATIC LIFE
RECOMMEND
CRITERIA
FED. GOV. WATER
AUTHORITIES
ICW ICE
AGREEMENT
PROV. GOV. WATER
AUTHORITIES
NATIONAL GUIDELINES
FOR
WATER QUALITY OBJECTIVES

PLAN FOR DEVELOPING NATIONAL GUIDELINE FOR
WATER QUALITY OBJECTIVES

Anderson*, P.D., H. Horovitch* and N.L. Weinstein**. 1979. Pollutant mixtures in the aquatic environment: A complex problem in toxic hazard assessment. Proc. Fifth Annual Aquatic Toxicity Workshop, Hamilton, Ontario, Nov. 7-9, 1978. Fish. Mar. Serv. Tech. Rep. 862, pp. 100-114.

ABSTRACT

The probable sources and magnitude of pollutant mixtures which concur coincidentally in aquatic ecosystems are discussed. Rationales that consider the unique problems posed by the multiple toxicities of such incidental mixtures are proposed.

An approach to the study of multiple toxicity based on "dose-response" curves and isobolograms is presented. The model is evaluated for its usefulness in predicting the lethal and sublethal potencies of mixtures. Furthermore, the scope of the model's application through a range in water hardness is examined. The empirical studies are limited to heavy metal mixtures but inferences are made as to the significance of the results for the management of Canada's natural water resources against the hazards of pollutant mixtures, in general.

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The toxicities of pollutant mixtures to aquatic biota do result in adverse effects which are often different from and/or greater than the toxicities of their respective constituents in pure solutions. For this reason, toxicologists have traditionally used bioassays to evaluate through the responses of test-organisms the lethal and sublethal potency of complex effluents. The results are expressed as a function of raw effluence or proportions thereof. This bioassay approach has been particularly useful in accessing pollutant mixtures which arise from point sources, i.e. "at the pipe".

However bioassay assessments of "at the pipe" effluence do not screen all of the pollutant mixtures which occur in the aquatic environment. Overlapping "zones of influence" of point source pollution and non-point sources such as atmospheric fallout and agricultural runoff appear to be two important means by which mixtures of pollutants may arise in situ within natural waters. Furthermore, the latter means of pollutant-mixture formation seems the only plausible explanation for the multi-contamination that has been documented in rivers and lakes of non-industrialized areas (Uthe and Bligh, 1971). The potential for such pollutant mixtures to occur coincidentally is emphasized by recent reports that non-point sources are presently contributing significantly greater amounts of pollutants to aquatic ecosystems than that deliberately discharged at effluent pipes (Table I)

Given the multitude of possible permutations and combinations between pollutants which concur in the environment, it would appear an impossible task to empirically identify and evaluate by bioassay all the resulting mixtures for their respective toxicities. As an alternative our approach to the apparent problem has been to explore

Table I. Magnitude of nonpoint pollution (after Barton, 1978).

ESTIMATED POLLUTANT CONTRIBUTIONS TO U.S. SURFACE WATERS: 1000's TONS/DAY

	Sediment	BOD	Nitrogen	Phosphorus	Acid Drainage (as $CaCO_3$)	Salinity
Total Point Source (after treatment, 1975)		11	4	--	1	35
Total Nonpoint Source	13,597	72	28	10	9	162

basic mechanisms that govern the physiological interactions between pollutants. Through this appreciation we are attempting to develop laboratory screening models that can be employed with reasonable ease to assess the toxicity of mixtures. Furthermore, we assume that the forms of multiple toxicity, (i.e. toxicity specific to mixtures) that are of particular concern to water quality management agencies are distinguished by, (1) potencies either greater than or equal to that which would be expected should each constituent contribute to the total effect in proportion to its respective potency, and/or (2) toxic effects which are different from and more insidious than the respective toxicities of each constituent of a mixture (Anderson and d'Apollonia, 1978).

We have included a traditional pharmacological rationale in our attempt to understand the mechanisms of multiple toxicity (Figure I). This concept distinguishes between pharmacokinetic and pharmacodynamic phases within which physiological interactions between individual pollutants may occur. Note that chemical interactions between discrete pollutants can arise in the environmental phase and may result in new toxic product(s). The latter phenomenon is not included in the subject of multiple toxicity unless there are eventual physiological interaction between the resulting chemicals.

Hazardous multiple toxicities arise in the pharmacokinetic phase from physiological interactions which affect a toxicant's compartmentalization and metabolism and which thereby alter the concentrations and/or forms of toxicants that reach receptor sites. In contrast, hazardous forms of multiple toxicity due to physiological interactions in the pharmacodynamic phase involve or follow from events at tissue receptor sites such as a toxicant's affinity and efficacy (Anderson and d'Apollonia, 1978).

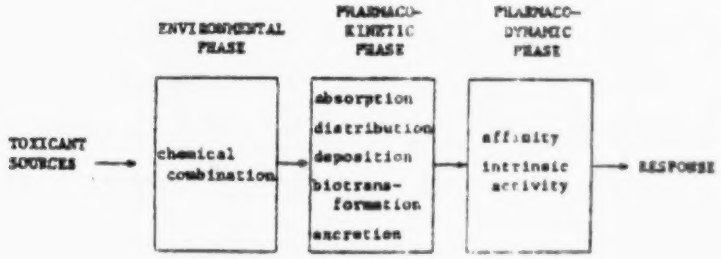
ENVIRONMENTAL PHASE
PHARMACO-KINETIC PHASE
PHARMACO-DYNAMIC PHASE
TOXICANT SOURCES
chemical combination
absorption
distribution
deposition
biotrans-formation
excretion
affinity
intrinsic activity
RESPONSE

Figure 1. Phases in which toxicants may interact. (Anderson and d'Apollonia, 1978)

The laboratory "modus operandi" which was followed for lethal and sublethal studies has been reported in previous papers (Anderson and Weber, 1975; Weinstein and Anderson, 1978). The easiest form of multiple toxicity to conceptualize and test empirically is based on the assumption that similarly-acting toxicants contribute to the end effect in proportion to their respective potencies as discrete agents. We call this model, STRICT ADDITION. Another "additive" model of multiple toxicity is called, RESPONSE ADDITION. Response addition is not deemed a particular hazard because, in accordance with its assumed mode of interaction, multiple toxicity effects should not occur when each of a mixture's constituent is below its respective toxic threshold. Therefore, water quality criteria which set safe limits based on studies of individual pollutants should be effective in preventing multiple toxicity risks due to RESPONSE ADDITION.

However, certain mixtures cause sublethal and lethal effects that are greater in magnitude than that predicted by the empirical models of STRICT and RESPONSE ADDITION. Such multiple toxicities are categorically termed SYNERGISMS and are assumed to be the result of pharmacokinetic and/or pharmacodynamic interactions (Anderson and d'Apollonia, 1978). At this stage in our investigations, we are unable to identify the specific mode of interaction which leads to a certain synergistic effect. Thus, we have opted to represent synergistic responses relative to that which would be expected empirically for STRICT ADDITION. Our denotation for this representation is SUPRA ADDITIVE SYNERGISM. It is important to understand that supra additive synergisms may or may not be the result of similarly acting substances. Reported herein are some of our findings gathered in studies of heavy metal mixtures.

As reported in last year's Workshop, copper and nickel mixtures at both lethal and sublethal levels were synergistic. At lethal levels the enhancement factor above STRICT ADDITION was more than two fold (Figure 2). At presumed sublethal levels the degree of supra additivity between copper and nickel virtually eliminated egg production and furthermore caused mortality within cultures during the second half of the ten-day reproductive studies (Figure 3).

Since then further analyses have shown that not only the presence but also the relative proportions of copper and nickel govern the degree

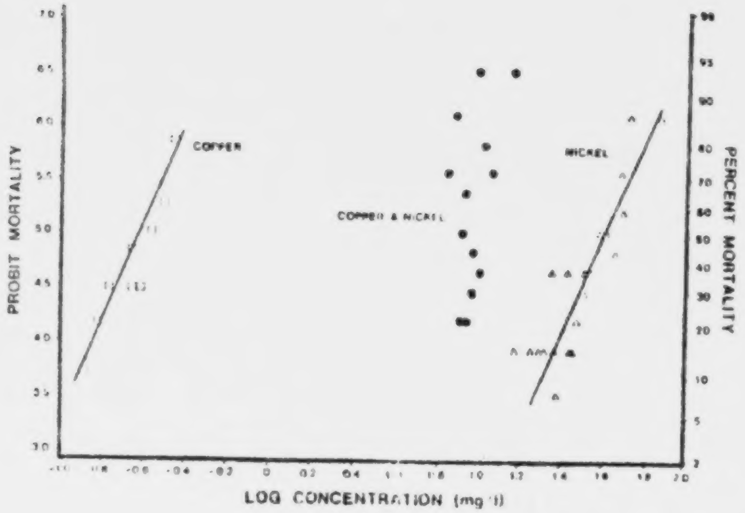

Figure 2. Lethal response data for zebrafish exposed to copper, nickel and their mixtures. The dose response line predicted by the model of STRICT ADDITION for Cu-Ni mixtures is the nickel line (Weinstein and Anderson, 1978).

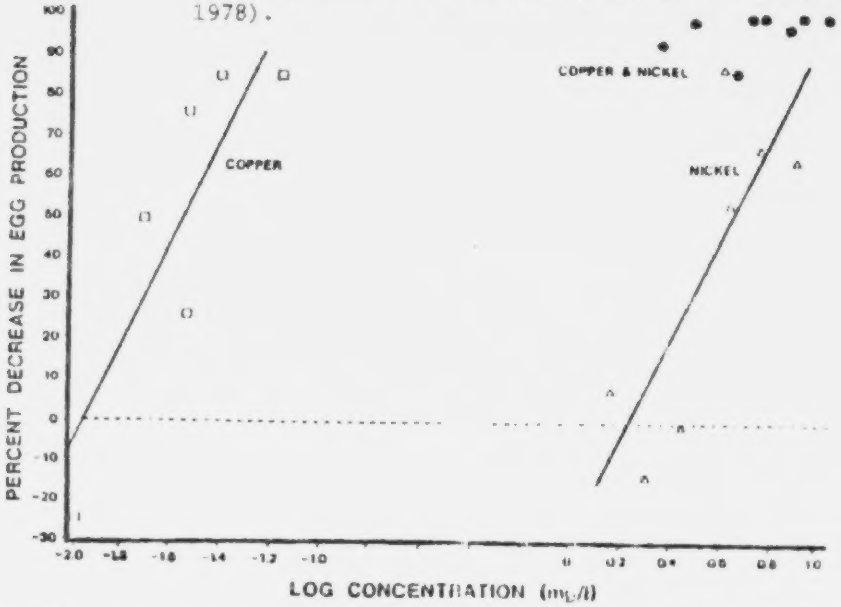

Figure 3. Sub-lethal response data for zebrafish exposed to copper, nickel and their mixtures. The dose response line predicted by the model of STRICT ADDITION for Cu-Ni mixtures is the nickel line (Weinstein and Anderson, 1978).

of synergism expressed by their mixtures. For example, Table II shows that when the proportion of nickel to copper is varied - while the total concentration is held constant - a difference in lethal potency is observed.

Table II. Effect of toxicant ratio on lethal response in zebrafish exposed to nickel and copper mixtures (Weinstein, 1978).

Total concentration of mixture (as Ni) in mg/l	Ratio $\frac{\mathrm{Ni}}{\mathrm{Ni\ Cu}}$	Observed % Mortality
8.68	.42	64
8.69	.81	21
9.48	.46	43
9.49	.82	29
10.68	.41	90
10.69	.84	79

We have evidence which shows that the relative proportion of constituents can be a major factor influencing the potency of mixtures of other substances. These findings add a new dimension to the already complex task of evaluating the effects of multiple toxicity. In consideration of this problem, Weinstein (1978) has developed a function that quantitates the shift in potency through a full range in proportions between copper and nickel. This quantitative function is graphically illustrated by the SUPRA ADDITIVE line in Figure 4 and represents, with reasonable precision, our empirical data for copper and nickel mixtures.

Another application of this quantitative function is shown in Figure 5. When the function is used to adjust for changes in potency due to proportions, we obtain a greater correlation to a linear regression for data shown previously in Figure 2.

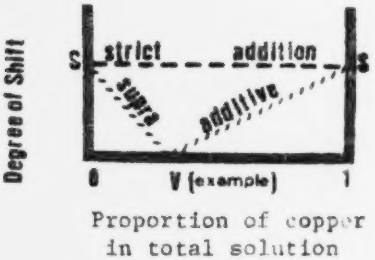
Degree of Shift
S
strict
addition
S
supra
additive
0
V (example)
1
Proportion of copper
in total solution

Figure 4. Isobologram depicting the differences in supra-additive potency due to relative proportions of copper and nickel in mixtures, where "V" represents the maximal effect (modified from Weinstein, 1978).

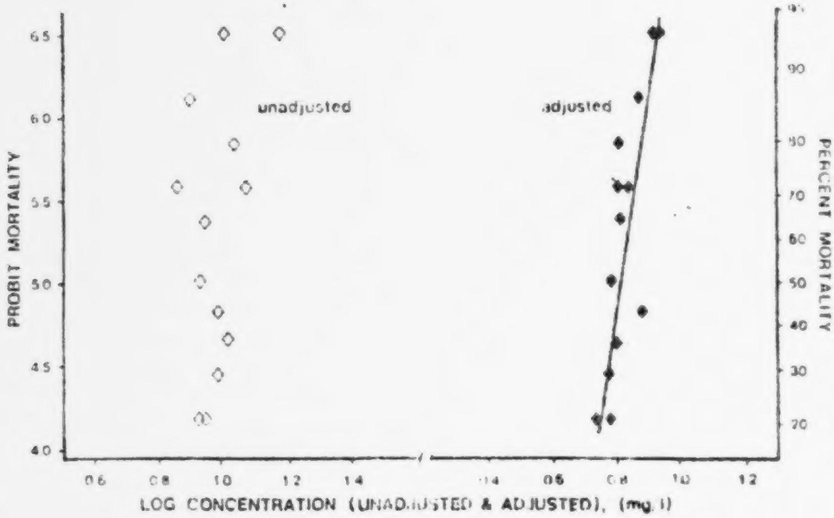

Figure 5. Dose response curves for unadjusted and adjusted lethal toxicity data for zebrafish exposed to mixtures of copper and nickel (Weinstein, 1978).

A second multiple toxicity project has been undertaken by Horovitch who studied the effects of water hardness, an environmental modifying factor, on the toxicity of binary mixtures of copper and zinc.

A survey of the literature suggests that the potency of many heavy metals varies with water hardness. Horovitch's investigations confirmed these reports by showing that the lethal potencies of copper and zinc, in pure solutions, decreased dramatically with increase in hardness as Ca^{++} and Mg^{++} ions (Figure 6). She showed that these relationships were not influenced by alkalinity because the latter ions were held constant between all hardness regimes which ranged from 20 to 300 ppm (expressed as $CaCO_3$).

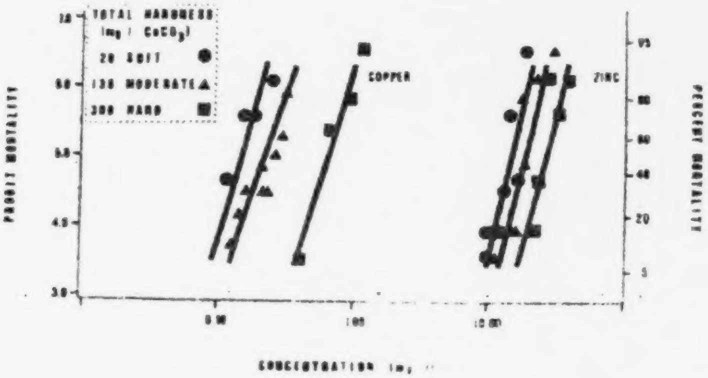

Figure 6. Lethal response data for zebrafish exposed to copper and zinc at three total water hardnesses.

In contrast to the studies of pure solutions, we found that the lethal potency of copper and zinc mixtures remained at virtually the same magnitude throughout the hardness regime (Figure 6). However if the data for their mixtures are represented relative to that expected for strict addition, we find that Cu-Ni mixtures are strictly additive in soft water and become pregressively more supra additive with hardness (Figure 7). Based on these results, the discrepancy between reports re. the mode of lethal toxicity of copper and zinc mixtures at different hardness regimes (Anderson and Weber, 1976) is simply a matter of the traditional manner in which the data has been compared. Allow us to reiterate this point. The SUPRA ADDITIVE synergism which characterizes copper and zinc mixtures in hard water is the consequence of changes in the potency of each metal in pure solution, not in the potency of their mixtures. The potency of their mixtures remained constant throughout the hardness range tested and the magnitude of that potency was predicted by the model of STRICT ADDITION applied to data recorded in the soft water regime.

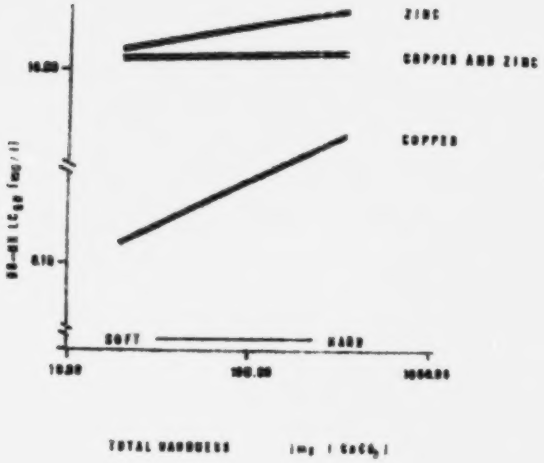

Figure 7. The effect of total water hardness on the 96-hr. LC50 of zinc, copper and their mixtures to zebrafish.

The implications of this apparent phenomenon are profound! Copper and nickel are often found together as contaminants of natural water. In these instances, any leniency in water quality standards to safeguard organisms for waters of high Ca and Mg levels is not justified.

Horovitch is presently studying the role that Ca and/or Mg^{++} ions play in the accumulation of copper and nickel in test fish.

Some of the preliminary results are depicted in Figure 8.

This illustration shows the relationship of hardness to copper's and zinc's content in gills following 48 hours of exposure to either copper or zinc alone or to their mixture. The results are inconclusive. Assuming the lethal potency for their mixtures is constant over the hardness range studied and assuming the gills are the critical site of lethal action, we did not expect to observe a change in the slope of the lines for either copper or zinc in gills of fish exposed to Cu-Zn mixtures. However, as can be seen in Figure 8, there is a decrease in the slope of these lines with hardness, although the magnitude of the change is not as great as for either metal in fish exposed to each substance discretely. Further analyses are being carried out.

Two other projects on the multiple toxicity of heavy metals are being conducted in our laboratory. One is investigating the patterns of multiple toxicity throughout the life cycle of an organism and the other is examining differences in multiple toxicity that may occur through time between accumulative and non-accumulative heavy metals.

Although these latter two projects have yet to be completed, we have found to date, that the patterns of multiple toxicity for cadmium and zinc would appear to differ between certain physiological stanzas within the life cycle. For example, conclusions based on studies which employ adult organisms would not apply, to those which test the fry stage. Furthermore, a non-accumulative heavy metal, cadmium, interacts with an accumulative heavy metal, mercury, long after that exposure time at which tolerance to cadmium, alone, developed in test organisms. These data seem to indicate that apparent safelevels of non-accumulative toxicants can enhance the toxicity of accumulative heavy metals. The implications are immense for the present approach of setting effective water quality criteria for heavy metals based on pure solutions only or on one life stage of test organism.

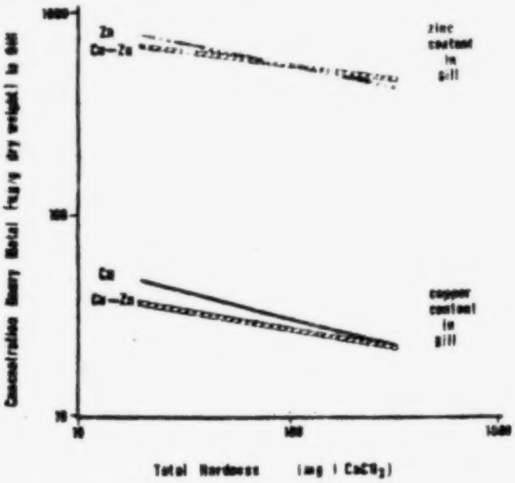

Figure 8. The effect of total water hardness on the copper and zinc content of gill tissues from zebrafish exposed as below:

48-HR Exposure Regime (mg/l)

copper	zinc	
.057	-	———
.025	3.000	▨▨▨
-	6.500	— · · — · ·

These last observations serve to reinforce our message that multiple toxicity is a unique, complex and significant problem. Thus the magnitude and apparent ubiquity of non-point source pollution in aquatic ecosystems necessitates an adjustment of the traditional approach to toxic hazard assessment. To begin with, we should revise the concept of the "mixing zone" which has referred to the receiving waters of point source effluents and which has been considered by some to be the only significant component of surface waters threatened by pollution. At some time in the past, this rationale may have been justified in that non-point pollution was not recognized as a significant pollutant source. Considering the vectors of non-point pollution, it is obvious that mixing zones can virtually involve any aquatic ecosystem. Therefore, it is no longer sufficient to rely solely on single contaminant toxicity testing or bioassays of "at the pipe" effluents. There is a need to expand Canada's water quality criteria program to seek means of assessing and preventing the toxic hazards of pollutant mixtures which arise coincidently in the environment.

Acknowledgements

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ABSTRACT

The deposition of air-borne acid is causing the acidification of susceptible lakes and rivers in south-central Ontario. Fish populations respond with altered growth, failed recruitment of age classes, loss of older animals, and drastic reduction in abundance. The acid stress may lead to loss of populations, loss of entire species and even the extinction of fishes from such waters. Many of the environmental correlates of this process which have been observed in the field, now need to be tested under controlled conditions.

Key words: Acid deposition, acid precipitation, lake acidification, acid stress, fish response, toxicology.

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INTRODUCTION

The phenomenon of acid precipitation has been reported widely in Europe and North America. Swedish scientists first observed that the average content of acid and sulfate was increasing in rain and snow. Oden (1968) collected the available data from western Europe and mapped the annual expansion of the area of acid precipitation. The magnitude of the Swedish problem was described in an official publication of the Swedish government in 1971. The cause of acid precipitation was attributed to the oxides of sulfur and nitrogen, transported long distances from their origin in Britain, France and Germany. The same problem was identified in Norway by Rosenqvist (1970), with the greatest effects in areas of already acidic soils. In 1972 the Norwegian Council for Scientific and Industrial Research, the Agricultural Research Council of Norway and the Norwegian Ministry of the Environment launched a large, joint research project "Acid precipitation - effects on forest and fish" (acronym: SNSF Project)

In North American, the acidification of surface waters was reported initially near major sources of sulfur dioxide emission (Gorham and Gordon, 1960; Gordon and Gorham, 1963; Beamish and Harvey, 1972; Conroy et al., 1974). This phenomenon was found to be more widespread than just near point sources (Likens and Bormann, 1974; Cogbill and Likens, 1974; Schofield, 1976; Dillon et al., 1977). Areas of northeastern United States, south-central Ontario, Quebec and the Maritimes contain waters which are especially low in alkalinity, and hence are susceptible to acid precipitation. This susceptibility is only now being determined accurately by Gran titrations for buffering capacity.

The effects on fish of low pH has been under laboratory study for many years. Much of this has taken the form of acidification of a domestic water supply and recording duration of exposure and time of death. The test conditions were complicated often by unknown concentrations of calcium and magnesium, which tend to protect against hydrogen ion, and carbon dioxide, which acts synergistically with H^+.

In the natural environment, acidification of lake and river waters has been observed to have many effects on fishes: changes in blood chemistry, physical deformity, recruitment failure, loss of older age classes, enhanced growth, retarded growth, observed mortalities, loss of populations, and complete extinction. Typically these observed effects are simply correlates of measured environmental variables, most notably pH. There remains the task of verifying these observations under controlled conditions.

BLOOD CHANGES AND PHYSICAL DEFORMITY

Acid stress has been found to cause disruption of the ionic composition of the blood, with fish showing the loss of about one-half of plasma sodium (Packer and Dunson, 1970; Leivestad and Muniz 1976). This effect of hydrogen ion on osmoregulation is antagonized by calcium ion (Lloyd and Jordan, 1964). Acid-stressed lakes are typically those with very low concentrations of calcium and hence the fish receive little protection from this source. In acid-stressed George Lake, female white suckers failed to show the normally elevated serum calcium associated with the maturation process (Beamish et al., 1975).

The George Lake suckers were exposed to an increasing concentration of hydrogen ion. For 1970 Beamish et al. (1975) reported that less than 1 per cent of the white suckers examined showed any deformity; by 1972, 32 per cent of the suckers examined were deformed. This phenomenon occurred also under laboratory conditions among white suckers maintained at low pH (Beamish, 1972), and among fathead minnows held over the range of pH 4.5-5.2 (Mount, 1973).

RECRUITMENT FAILURE

The author observed the white suckers in acid-stressed Lumsden Lake in the spring of 1966. Spawning appeared to be unsuccessful. A sample of the population was analyzed for age composition (Beamish, 1970) and again in 1967. The result showed an absence of 5-year-old fish in the population. The near-absence of age classes 0 and 1 from the population was further evidence of recruitment failure (Fig. 1). This phenomenon has occurred in other species in other lakes. The rock bass populations in lakes Carlyle, Bell, Johnnie and Owl were composed of fish 5 years old and older (Ryan and Harvey, 1977). These lakes are among the most severely acid-stressed in the La Cloche area, but which still contained rock bass. The yellow perch of Patten Lake showed the same effect (Fig.2) with few fish caught younger than six years (Ryan and Harvey, submitted).

In a study of 25 La Cloche lakes containing rock bass, Ryan and Harvey (1977) found age-group 0 least often was present in lakes of low pH (4.0-4.5) and most common in lakes of high pH (6.0-6.5). The same was true for yellow perch in 39 lakes (Ryan and Harvey, submitted).

There remains to be identified the mechanism or mechanisms by which this recruitment failure is brought about. It may be through failed maturation, failed spawning behaviour, the death of fish larvae post-hatching, or such like. There is a small amount of observational evidence in support of each of these in different species of fish.

However, whatever the mechanism, the effect of prolonged severe acid stress is the same. The population rises in average age, is reduced to a few old individuals, and eventually becomes extinct.

The antithesis of the above process is the loss of older age classes from some fish populations in acid-stressed lakes. The George Lake population of white suckers had individuals up to 14 years (Beamish, 1970) in 1967, but by 1972, (Beamish et al., 1975) there were almost no suckers found older than 6 years; eight age-classes had been lost (Fig. 3).

CHANGES IN GROWTH

Decreased growth rates for fishes in acid waters have been reported by several authors. Frost (1940) found older trout growing more slowly than younger ones. Yellow perch in acid-stressed Swedish lakes grew more slowly (Almer, 1972), possibly in response to reduced food supply. Beamish et al. (1975) reported the slower growth of white suckers in George Lake, as it acidified, 1967 to 1972. Yellow perch in 39 La Cloche Lakes grew more quickly at low pH up to age 3 years, but thereafter grew more slowly (Ryan and Harvey, submitted).

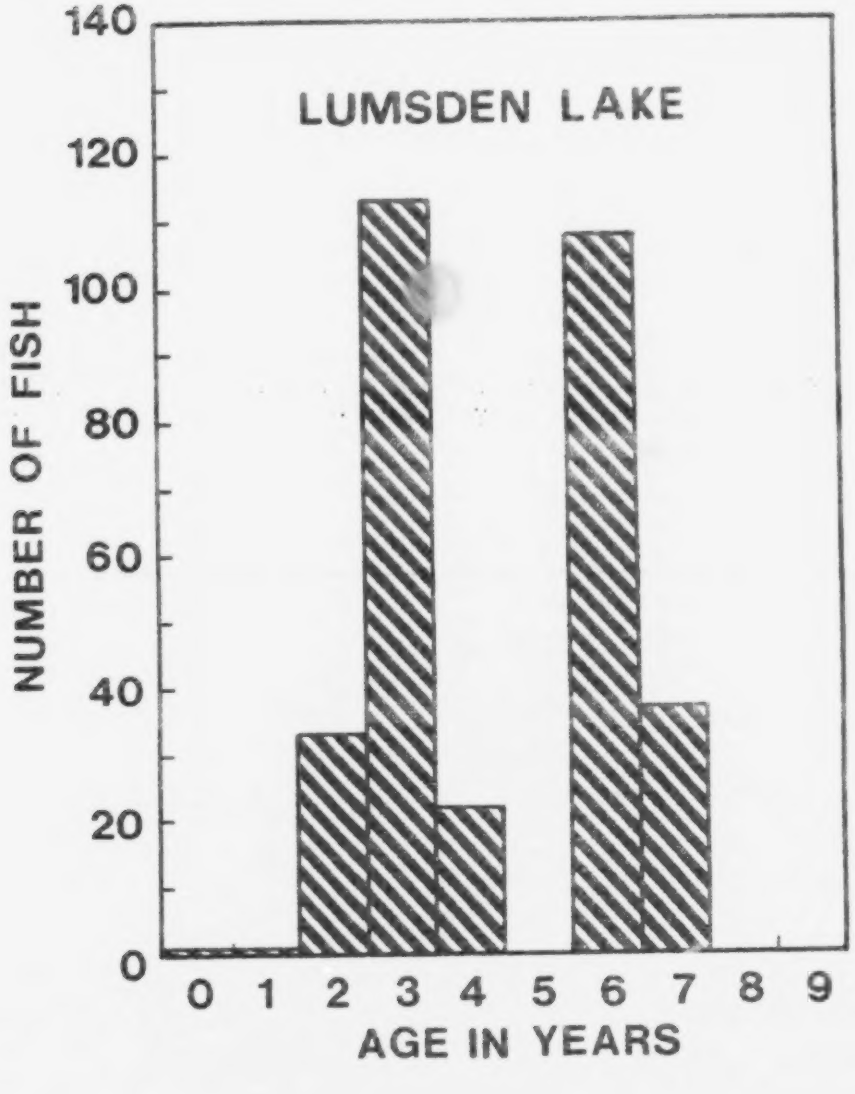

Figure 1. Age composition of white suckers captured in Lumsden Lake. (Based on Beamish, 1970).

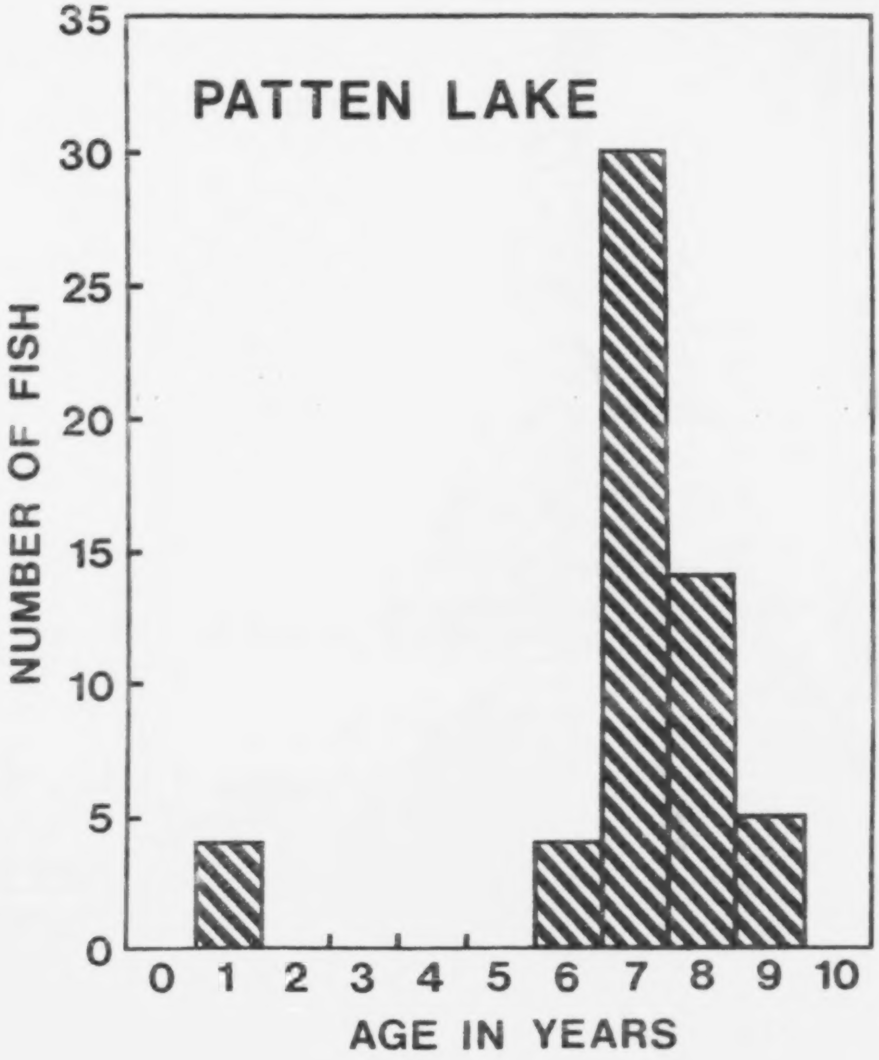

Figure 2. Age composition of yellow perch captured in Patten Lake. (Based on Ryan and Harvey, submitted).

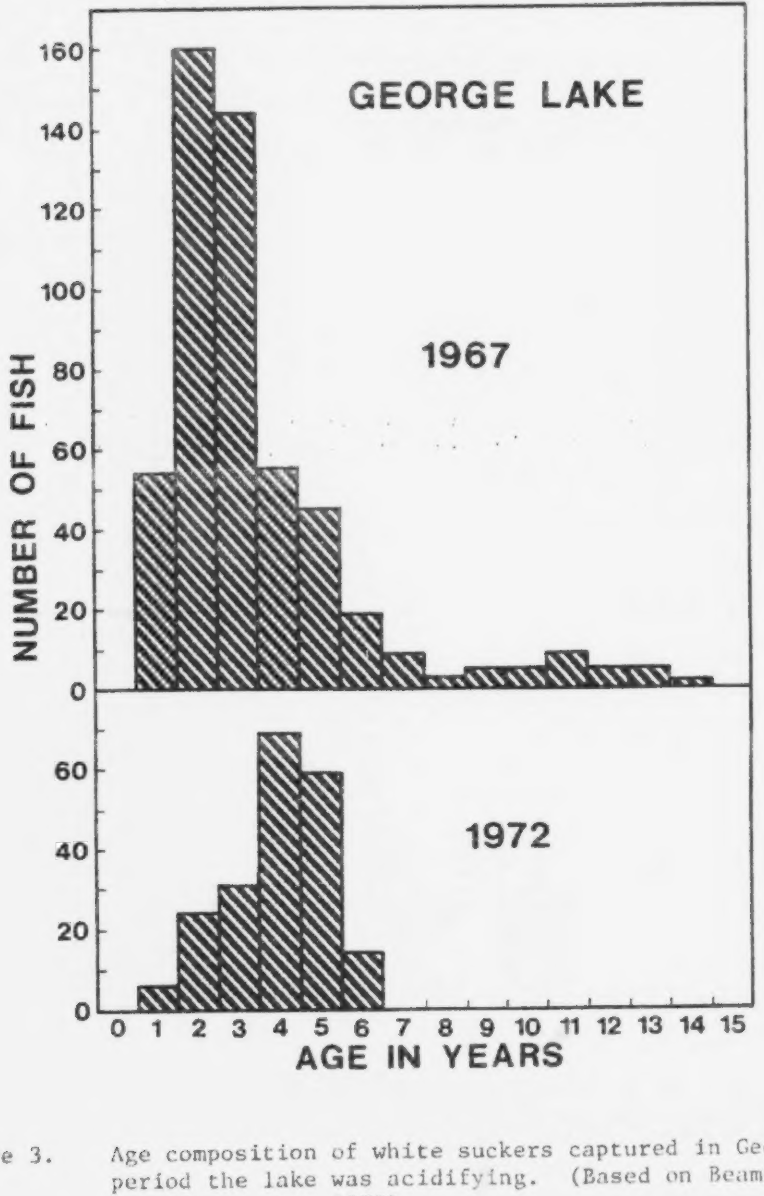

Figure 3. Age composition of white suckers captured in George Lake, over the period the lake was acidifying. (Based on Beamish, 1970 and Beamish et al., 1975).

It is possible that decreased growth rate could result from either the direct effect on fish metabolism or from the indirect effect of a reduced food supply. An acid stress on fishes results in an ionic imbalance, and combating this stress requires the expenditure of energy, possibly at the expense of growth. The top predators are among the more acid-tolerant species, and many prey species,especially the cyprinids, are less tolerant of low pH. The yellow perch, being among the most acid-tolerant, survives in acid-stressed lakes, often in the absence of prey species. Ryan and Harvey (submitted) postulated that this was the cause of the reduction in growth of perch older than three years. For this reason Ryan (1976) distinguished between piscivorous and non-piscivorous fishes in response to acid stress.

Increased growth rates of fishes under acid stress have been reported several times. Parsons (1952) found this in bluegill, Almer (1972) in roach, and Jensen and Snekvik (1972) in salmonids. Rock bass were larger at low pH (Fig. 4) over the age range 1 to 8 years (Ryan and Harvey, 1977). This increased growth can be attributed to increased food supply as a result of reduced interspecific competition for food. The main food of the rock bass is invertebrates, and many invertebrates species survive concentrations of hydrogen ion which are lethal to fish. Rock bass are among the most acid tolerant of temperate freshwater fishes, and as such survive acid stress beyond their competitors. Intraspecific competition may also be reduced, for example through failure of recruitment. The result is a very small population of relatively old, sometimes fast-growing, acid-tolerant individuals. In such populations, intensive sampling may yield only a very small sample, for example, the total catch of rock bass was 11 fish in Carlyle L., 13 in Kilarney L., 24 in Johnie L., 32 in Owl L. (Ryan and Harvey, 1977). Beamish (1974) intensively netted O.S.A. Lake and captured only 4 yellow perch, 2 rock bass, and 8 lake herring. Study of such vestigial populations is very difficult.

LETHAL EFFECTS

Dead and dying fishes are seldom reported from acid-stressed lakes. The mechanisms of mortality, reproductive failure, loss of larvae, and longest survival by top predators, preclude easy direct observations. Fish kills have been observed in rivers, for example during an acid pulse. Leivestad and Muniz (1976) reported a massive kill in the Tovdal River in Southern Norway. This coincided with exceptionally low pH during the early phase of snow melt. Thousands of brown trout were killed over a length of 30 km of river; the Tovdal was once a salmon river. Harvey and Zimmerman (1978) observed a number of dead and dying fish, especially pumpkinseed, in Plastic Lake, coincident with spring run-off entering the lake. The lake surface showed a pH of 5.5 and the major inlet stream was pH 3.8 (Fig. 5).

The loss of fish species from lakes and rivers has been reported many times from Norway, Sweden, Canada and United States. These reports tend to minimize the extent of such losses, in that often the fish communities of lakes is now known from the pre-stress period. This is especially true of remote lakes. Schofield (1976) for example had records of fish presence in forty Adirondack Mountain lakes above 610 meters from 1929-1937 and was able to demonstrate the loss of fishes to 1975 from many of these lakes. Beamish and Harvey (1972) lacked similar surveys from the La Cloche Mountain Lakes. Most known losses of fish populations were based on earlier angling success (Fig. 6). Intensive surveys of 68 lakes yielded 19 lakes with no fish present (Harvey, 1975) and about half of the remaining lakes showed very modest species associations. Beamish (1974) reported on the loss of the sport fishery for lake trout and smallmouth bass in O.S.A. Lake.

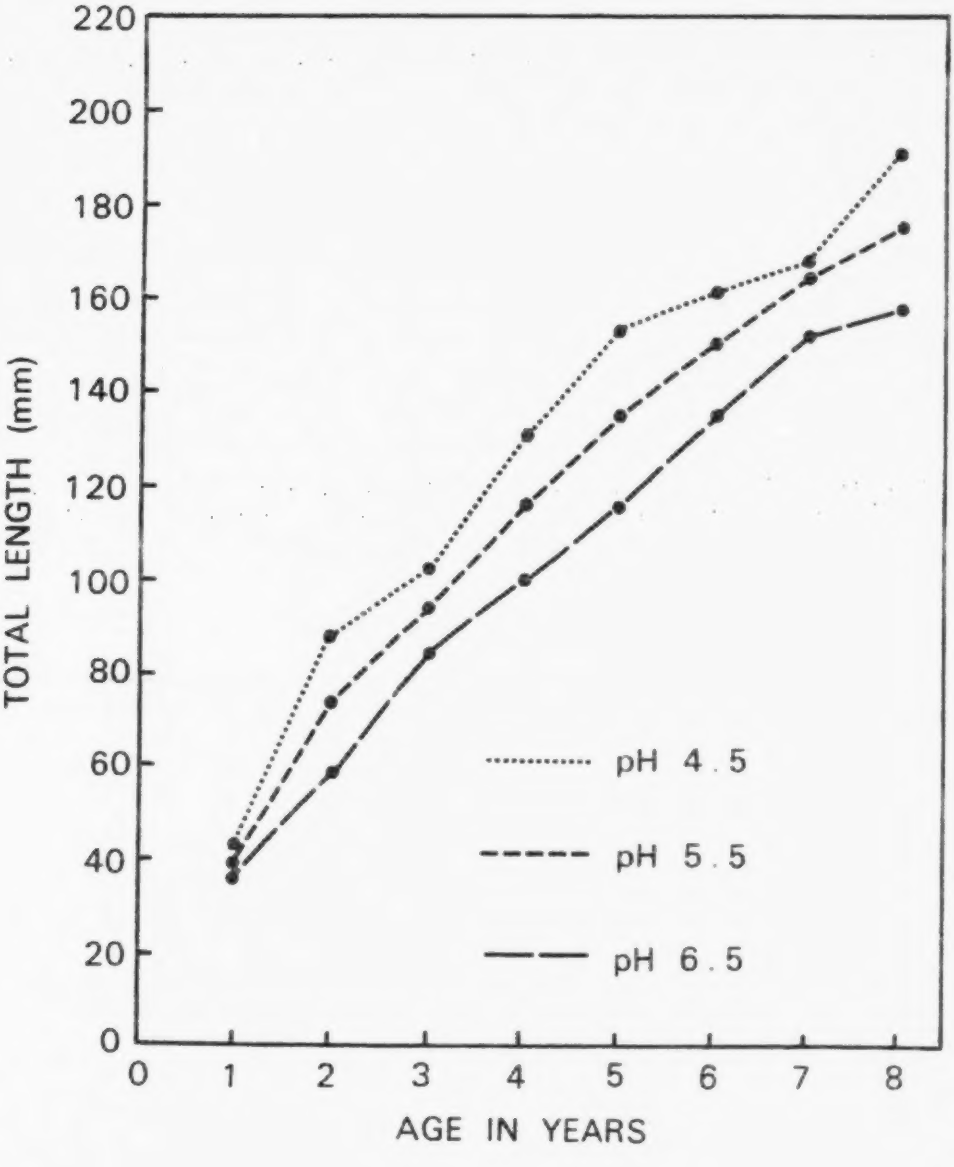

Figure 4. Size and age of rock bass in relation to pH in 25 La Cloche Lakes. (Based on Ryan and Harvey, 1977).

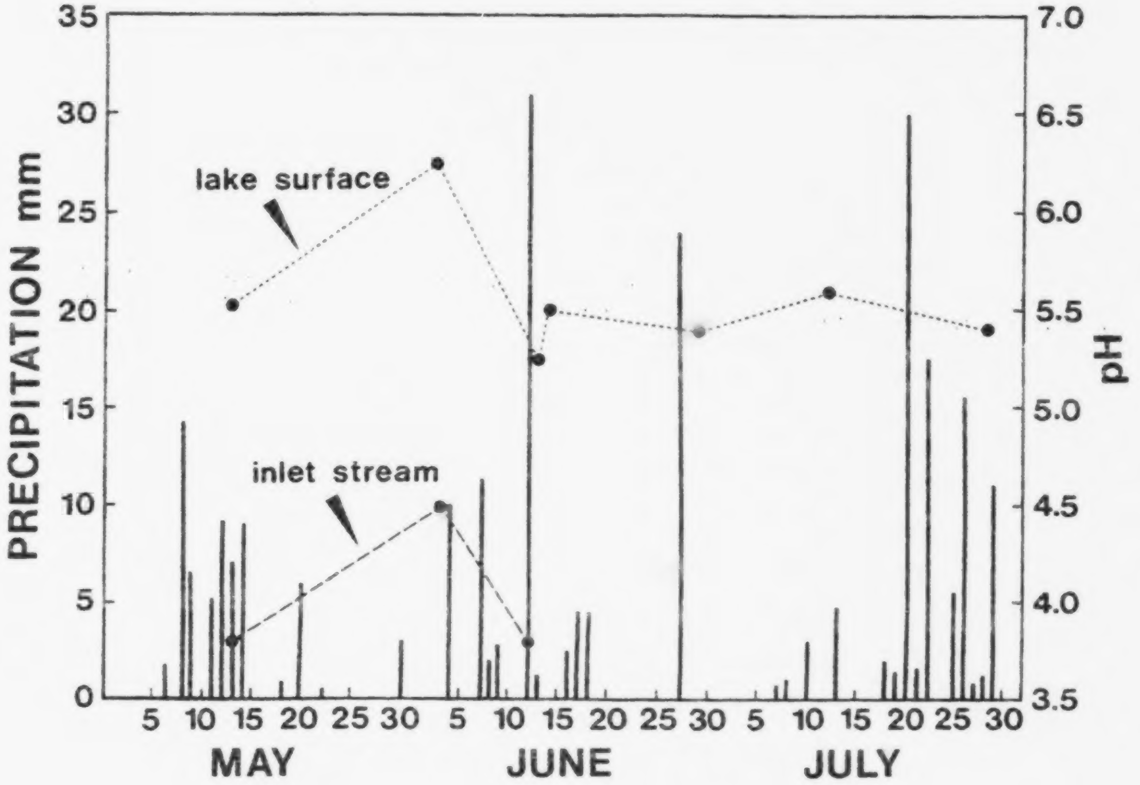

Figure 5. Surface water and inlet stream pH of Plastic Lake, Spring 1978, coincident with a significant fish kill. Vertical bars are precipitation amounts. (Based on Harvey and Zimmerman, manuscript).

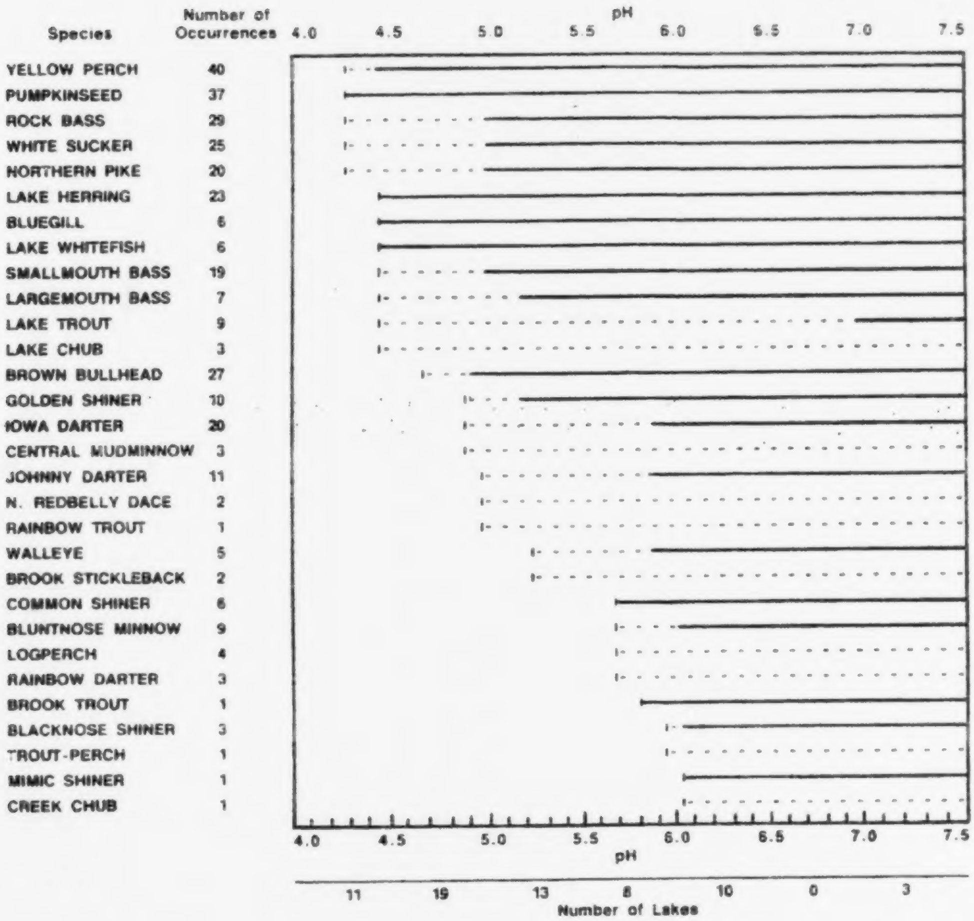

Figure 7. Incidence of occurence of fish species in the La Cloche Mountain Lakes of Ontario, in relation to pH. Solid line represents populations apparently unaffected by acid stress. Dashed line indicates populations showing stress, based on age-class structure, growth changes, etc., or where the sample taken was too small for diagnostic purposes. Vertical bar marks the lowest pH recorded for the species. (Harvey, unpublished MS).

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ABSTRACT ONLY

Water pollution by cyanide wastes mainly occurs as a result of mining and industrial operations. Fish may encounter a wide range of cyanide concentrations at different water temperatures due to daily fluctuations, seasons, latitude and thermal dishcarges. The influence of temperature on aquatic organisms has been widely studied but temperature as a modifying factor of toxicity has been given only limited attention in establishing water quality criteria.

The toxicity of cyanide (HCN) to juvenile rainbow trout (*Salmo gairdneri*) acclimated for three weeks to 6, 12 and 18°C was determined at these temperatures by flow-through bioassays, in the cyanide concentration range of 0.018 - 0.087 mg L^{-1}. The 96-hour median lethal concentrations (LC50) were 0.028 ± 0.004 mg L^{-1} at 6°C, 0.042 ± 0.004 mg L^{-1} at 12°C and 0.068 ± 0.004 mg L^{-1} at 18°C. Warm acclimated rainbow trout survived longer in lethal concentration of cyanide, although the difference in survival time decreased at higher concentrations tested.

Earlier research on the survival of trout in cyanide concentrations tested above 0.10 mg L^{-1} HCN indicated that trout were more susceptible to cyanide at higher temperatures. Therefore toxicity curves relating median survival time to a range of cyanide concentrations from 0.02 - 0.20 mg L^{-1} HCN at 6, 12 and 18°C were constructed using data of the present investigation as well as from other researchers. The relationship obtained clearly showed that the acute toxicity of cyanide to rainbow trout at different temperatures is concentration dependent. At slowly lethal concentrations cyanide is more toxic at lower temperatures whereas at rapidly lethal levels the reverse occurs. The reversal in the effect of temperature on toxicity of cyanide takes place at 0.10 mg L^{-1} HCN.

This reversal phenomenon has been observed by other authors as well, notably with zinc and phenol, both of which seem to kill fish by reducing the availability of oxygen to the tissues. It seems that at rapidly lethal concentrations, mortality from cyanide and other respiratory depressant poisons depends solely on the rate at which the toxicant reaches the active sites since ventillation and metabolic rates are faster at higher temperatures. At slowly lethal concentrations, the biochemical state of the fish through detoxification and excretory processes may enable rainbow trout to survive better at the warmer temperatures.

Canadian inland waters exhibit wide differences in temperatures due to seasonal and latitudinal factors, yet toxicants rarely enter water systems at rapidly lethal concentrations. It appears imperative to evaluate pollutants at realistic concentrations and at ambient temperatures likely to prevail in the natural environment.

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ABSTRACT

Concentrations resulting in lethality following short-term exposures to copper may be described by equations having the general form:

$$LC50 = aH^b$$

where a and b are empirical parameters and H is water hardness. Distinct equations of the above form were developed for the order Perciformes (perch-like fishes), the order Cypriniformes (minnow-like fishes), the family Salmonidae (salmon-like fishes; excepting Pacific salmon) and the genus *Oncorphynchus* (Pacific salmon). The LC50-hardness relationships are briefly discussed with reference to chronic exposure and the influence of other modifying factors.

At the sublethal level of exposure, threshold concentrations for the reduction of growth were found to increase with water hardness for representative species of the family Salmonidae and the order Cypriniformes. The thresholds for growth reduction in freshwater fish are compared to other sublethal thresholds.

RÉSUMÉ

La tolérance léthal aiguë des poissons exposés au cuivre peut-etre décrite par des équations sous la forme générale de:

$$CL50 = aH^b$$

dans laquelle a et b sont des paramètres empiriques et H est la dureté de l'eau. Des équations distinctes de cette même forme furent développées pour l'ordre Perciformes (poisons du type perchaude), l'ordre Cypriniformes (poissons du type méné), la famille Salmonidae (poisons du type saumon: à l'exception des saumons du Pacifique) et le genre *Oncorhynchus* (saumons du Pacifique). Les relations CL50-dureté de l'eau sont discutées brièvement avec référence à l'exposition chronique ainsi qu'à l'influence d'autres facteurs modifiant.

Au nineau sous-léthal d'exposition, il fut constaté que le seiul des concentrations nécéssiare à la réduction de la croissance augmentait avec la dureté de l'eau pour certaines expèces représentatives de la famille Salmonidae et de l'ordre Cypriniformes. Les seuils réduction de croissance chez les poissons d'eau douce sont comparés à d'autres seuils sous'lèthals.

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INTRODUCTION

A wide range of toxicologically-effective concentrations pertain to copper in the aquatic environment. With respect to freshwater fish, numerous acute toxicity tests conducted at the lethal level of exposure have confirmed a variation in response spanning more than two orders of magnitude. Even though standard procedures were adhered to, LC50s reported in the literature have varied from 0.010 mg/l Cu to 2.6 mg/l Cu. Within this concentration range certain factors may be identified which minimize the variation in lethal tolerance.

Some of the variation may be attributed to interspecific differences in tolerance. For example, perch-like fishes belonging to the order *perciformes* are most tolerant. Pacific salmon belonging to the genus *Oncorhynchus* are least tolerant. Morphological or physiological characteristics responsible for interspecific differences in tolerance have not yet been elucidated.

For each of the taxonomic groups of fish studied to date, the LC50s increase with increasing water hardness. Concentrations of total copper which result in lethality following short-term exposures may be described by equations having the general form:

$$LC50 = aH^{b} \qquad \text{(i)}$$

where a and b are empirical parameters and H is water hardness having the units mg/l $CaCO_3$. Such a relationship was proposed for the acute response of rainbow trout, *S. gairdneri*, exposed to copper (Lloyd and Herbert, 1962). A plausible explanation for the influence of water hardness is that calcium and magnesium ions may act intrinsically upon cell membrane permeability of the biological receptor (Lloyd 1962; Hoar 1969).

INTERRELATION OF LC50 AND WATER HARDNESS

Distinct equations of the above form were developed for different taxonomic groups of freshwater fish. For perch-like fishes belonging to the order *perciformes*, the LC50s vary from 0.66 mg/l Cu in soft water to 2.55 mg/l Cu in hard water according to the equation:

$$\text{96-h } LC50_{(mg/l\ Cu)} = 0.13\ H^{0\cdot 5}_{(mg/l\ CaCO_3)} \qquad \text{(ii)}$$

The relationship described by equation (ii) is presented graphically in Figure 1 in which each set of co-ordinates represents a single 96-h LC50 reported in the literature.

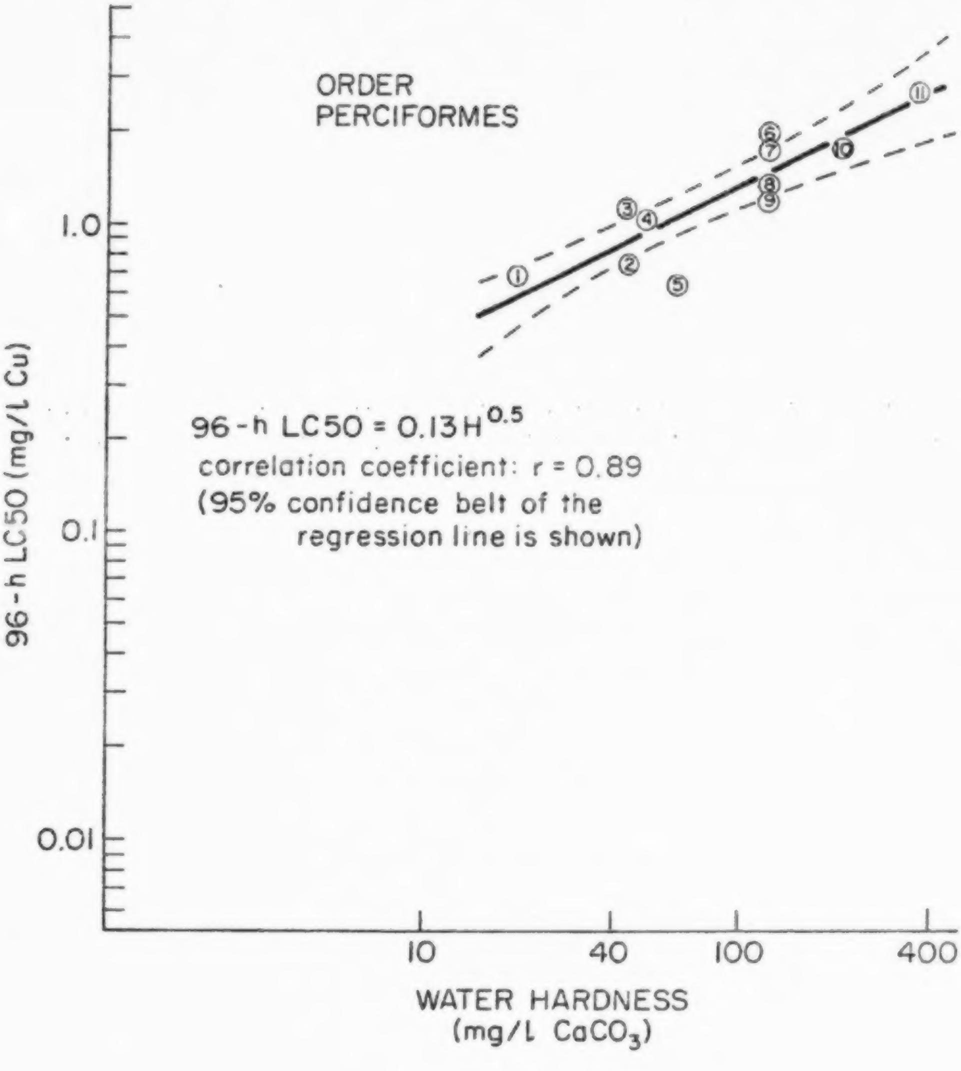

Figure 1: Relationship between short-term lethal tolerance of the order *perciformes* and water hardness.

For minnow-like fishes belonging to the order *cypriniformes*, the 96-h LC50s vary from 0.023 mg/l Cu in soft water to 1.76 mg/l in hard water according to the equation:

$$96\text{-h } LC50_{(mg/l\ Cu)} = 0.00085\ H^{1.22}_{(mg/l\ CaCO_3)} \qquad \text{(iii)}$$

The relationship described by equation (iii) is shown graphically in Figure 2. Equation (iii) may not apply to catfish, which belong to the family *ictaluridae*. Brown bullheads, *I. nebulosus*, were reported to have a 96-h LC50 of 0.18 mg/l Cu in water of 200 mg/l $CaCO_3$ hardness (Brungs *et al*. 1973; Christensen *et al*. 1972). However, the LC50 for the same $CaCO_3$ hardness is 0.54 mg/l according to equation (ii). Thus, the family *ictaluridae* may prove to be less tolerant than other *cypriniformes*. No satisfactory explanation has yet been found for this anomaly.

Unlike equations developed for the orders *perciformes* and *cypriniformes*, equations describing the interrelation of LC50 and water hardness for *clupeiformes* incorporate toxicity tests ranging from 48 hours to 21 days. Within the order *clupeiformes*, separate equations were developed for the family *salmonidae*, and the genus *Oncorhynchus*. For trout and Atlantic salmon belonging to the family *salmonidae*, LC50s vary from 0.025 mg/l Cu in soft water to 1.10 mg/l in hard water according to the equation:

$$LC50_{(mg/l\ Cu)} = 0.0034\ H^{0.91}_{(mg/l\ CaCO_3)} \qquad \text{(iv)}$$

For Pacific salmon belonging to the genus *Oncorhynchus*, LC50s vary from 0.010 mg/l Cu in soft water to 0.125 mg/l Cu in hard water according to the equation:

$$LC50_{(mg/l\ Cu)} = 0.0014\ H^{0.79}_{(mg/l\ CaCO_3)} \qquad \text{(v)}$$

The relationships described by equations (iv) and (v) are presented graphically in Figure 3.

Equations (ii) - (iv) delineate quantitatively the reported range in LC50s - from 0.010 mg/l Cu to 2.6 mg/l Cu. The relationships described by these equations have been plotted together (Figure 4) in order to illustrate the interorder differences in tolerance at various levels of water hardness. As seen in Figure 4, the order *perciformes* is most tolerant. The relative tolerance of the family *salmonidae* is similar to that of the order *cypriniformes*. The genus *Oncorhynchus* is least tolerant.

APPLICABILITY TO DIFFERENT SPECIES OF FRESHWATER FISH

For a given species belonging to one of these taxonomic groups, the lethal tolerance may be estimated at any water hard-

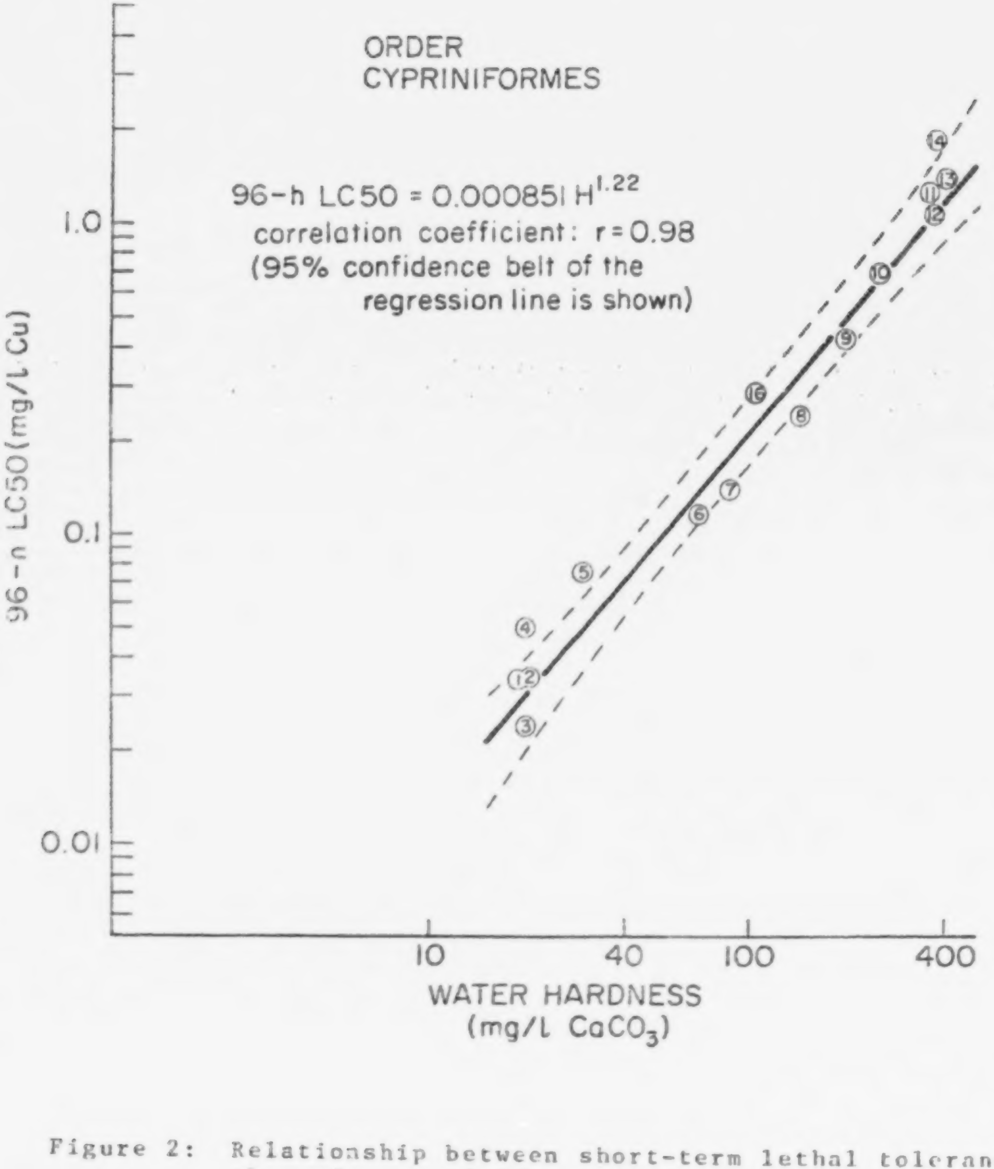

Figure 2: Relationship between short-term lethal tolerance of the order *cypriniformes* and water hardness.

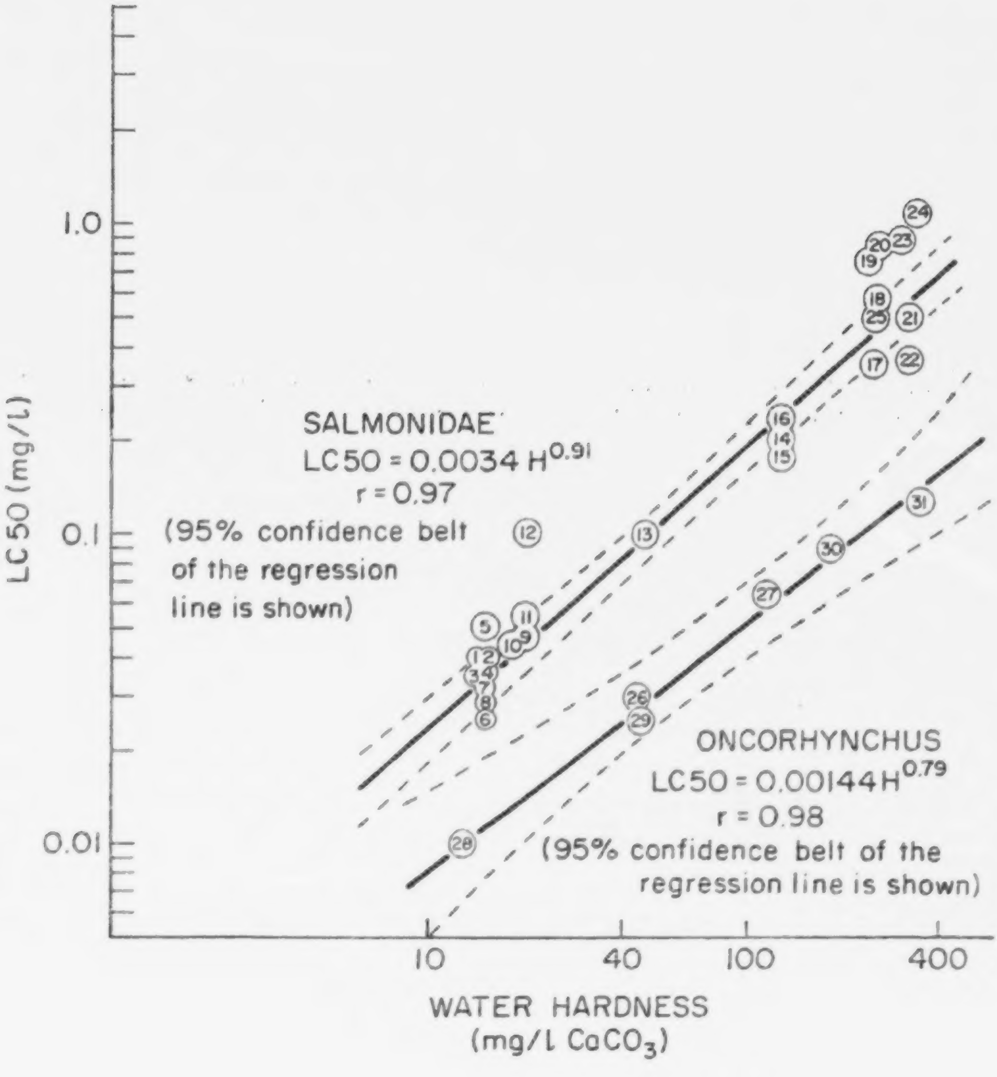

Figure 3: Relationship between lethal tolerance of the family *salmonidae* and the genus *Oncorhynchus* (both of the order *clupeiformes*) and water hardness.

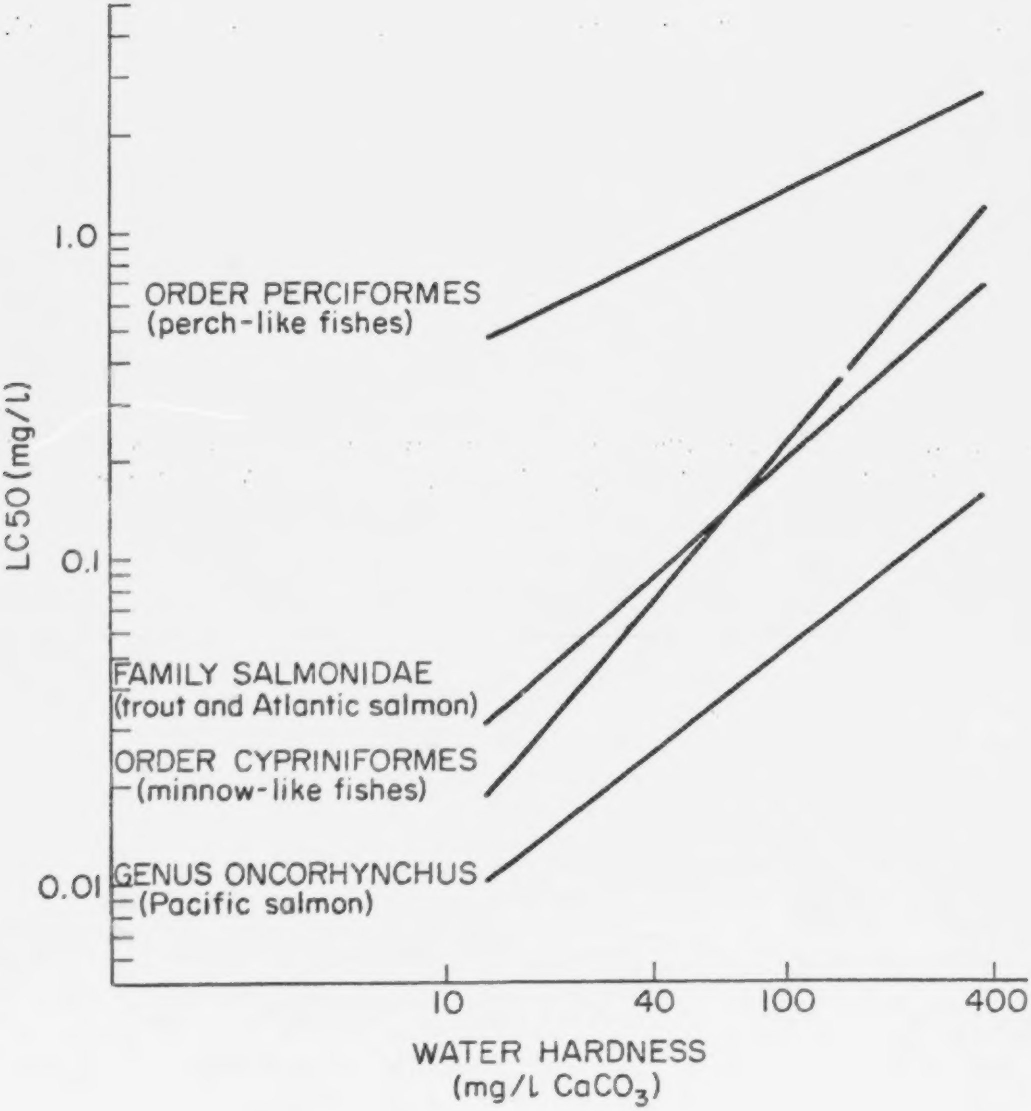

Figure 4: Interorder comparison of lethal tolerance of freshwater fish to copper with respect to water hardness.

ness. Thus, use of equations (ii) - (v) may have a predictive capability in certain circumstances. Studies into the lethal tolerance of freshwater fish have concentrated upon a few representative species, such as bluegills and rainbow trout, while the tolerances of other important species have not been verified. For Canada's principal commercial species (Scott and Crossman, 1975), including four species within the order *perciformes* and two species within the family *salmonidae*, LC50s have not been determined. Equations (ii) and (iv) may be applied with caution to commercial species of these taxonomic groups.

APPLICABILITY TO DIFFERENT NATURAL WATERS

Equations (ii) - (v) are presumed to be accurate estimations of the acute LC50 for fish inhabiting natural waters which are relatively free of complexing and adsorbing agents. However, these equations may underestimate the LC50 in situations where additional physico-chemical modifying factors decrease the toxic potency of copper.

Inorganic complexation by phosphates is known to reduce the toxic potency of copper (Andrew, 1976). Toxic potency is expected to decrease as pH and alkalinity increase due to inorganic complexation of the aquo cupric ion with hydroxo and carbonato ions. Equations (ii) - (v) include some variation due to pH and alkalinity; however, the relative influences of pH, alkalinity and water hardness are difficult to discern. Stiff (1971) proposed that alkalinity might replace water hardness as a major modifying factor. However, by maintaining pH and alkalinity at constant values, Inglis and Davis (1972) were able to demonstrate the influence of total hardness, *per se*, upon the tolerance of bluegills, *L. macrochirus* (data points included in equation (ii)). Furthermore, water hardness is used here as a basis for data comparison because alkalinity has frequently been omitted from published toxicologic reports.

Organic complexation may result in significant decrease of copper's toxic potency in soft water. However, due to Ca^{+2}, Mg^{+2} competition with Cu^{+2}, the influence of organic ligands is expected to diminish with increasing water hardness. The modifying influence of humic acid is shown in Figure 5 for different levels of water hardness. In addition to humic acid, organic chelators known to influence the tolerance of freshwater fish to copper include glycine, EDTA, and NTA. Citrate, which forms copper complexes of relatively low stability constants and cysteine, which forms monodentate cuprous complexes, may not alter the toxic potency (Black, 1974).

Adsorption of copper onto colloidal dispersions of clays and hydrous metal oxides as well as adsorption onto suspended particulate matter may decrease the toxic potency of copper to freshwater fish.

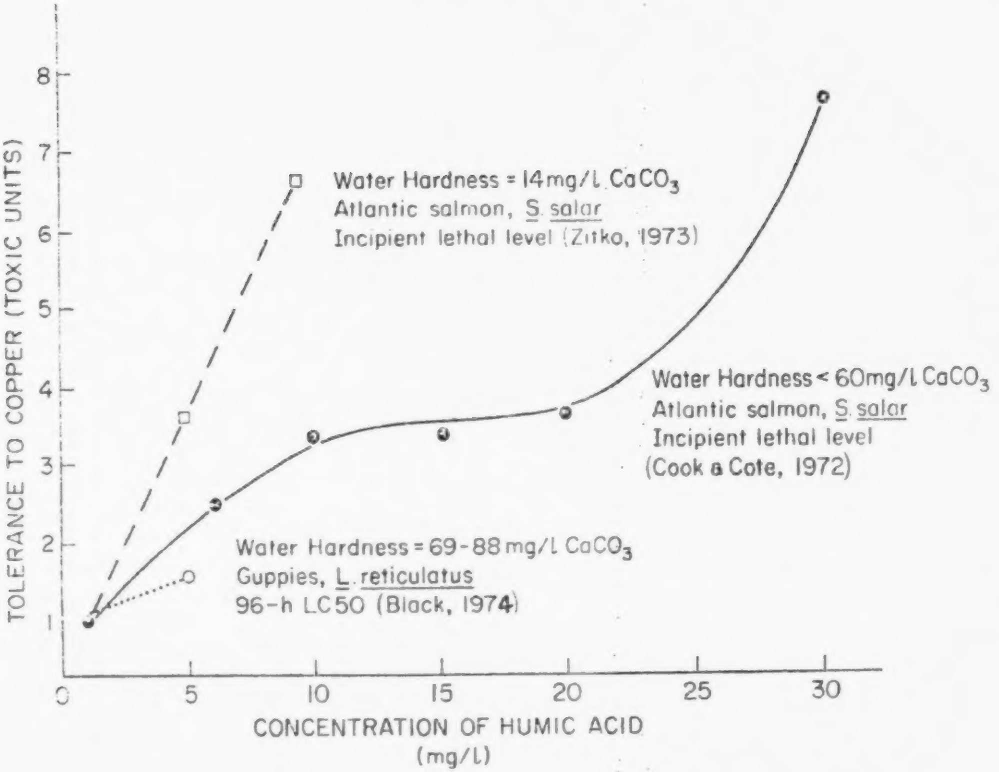

Figure 5: Effect of humic acid upon the lethal tolerance of freshwater fish to copper.

Biological modifying factors which may influence lethal tolerance include the physiological stage of development, pre-exposure to copper, and possibly the rate of gill ventilation. Freshwater fish in the post-larval stage of development are most sensitive to acute exposures of copper (McKim *et al.* 1978). Fish which have been pre-exposed to sublethal levels of copper may demonstrate resistance during acute lethal exposures (Dixon, personal communication). Gill ventilation rate may be increased by low oxygen levels, increased ambient temperatures, and increased swimming activity. Lethal tolerance is thought to be inversely related to gill ventilation rate (Lloyd, 1962).

SHORT-TERM VERSUS LONG-TERM EXPOSURES

Equations (ii) - (v) may overestimate the LC50 for fish exposed chronically to copper. For fathead minnows, *P. promelas*, exposed to copper in soft water, an approximation of the 60-day LC50 was found to be 0.2 of the 96-h LC50 (Mount, 1968). For fish of the same species in hard water, an approximation of the 11-month LC50 was found to be 0.36 of the 96-h LC50 (Mount and Stephan, 1969). In yet another study, the 63-day LC50 for stoneloach, *N. barbatulus*, was found to be 0.36 of the 96-h LC50 (Solbé and Cooper, 1976).

INTERRELATION OF GROWTH REDUCTION THRESHOLD AND WATER HARDNESS

At the sublethal level of exposure, growth reduction is as sensitive an indication of detrimental effects due to copper as are reproductive and developmental impairment. Furthermore, growth reduction is more sensitive a response than LC50s for fish in the post-larval stage of development.

Threshold concentrations resulting in growth reduction have been plotted against water hardness in Figure 6. Interorder differences in tolerance as well as changes in tolerance with water hardness are similar to those found at the lethal level of exposure. For a *perciform* species, the growth reduction threshold is 0.077-0.162 mg/l Cu in soft water (Benoit, 1975). For a *cypriniform* species, the growth reduction thresholds range from 0.011-0.019 mg/l Cu in soft water (Mount and Stephan, 1969) to 0.033-0.095 mg/l Cu in hard water (Mount, 1968). For species belonging to the family *salmonidae*, growth reduction thresholds range from 0.015 mg/l Cu in soft water to 0.100 mg/l Cu in hard water at pH 7.5-8.0 (Waiwood, 1977). However, copper may be more potent at low pH. At pH 6.0, growth reduction thresholds for the family *salmonidae* range from 0.010 mg/l Cu in soft water to 0.050 mg/l Cu in hard water (Waiwood, 1977).

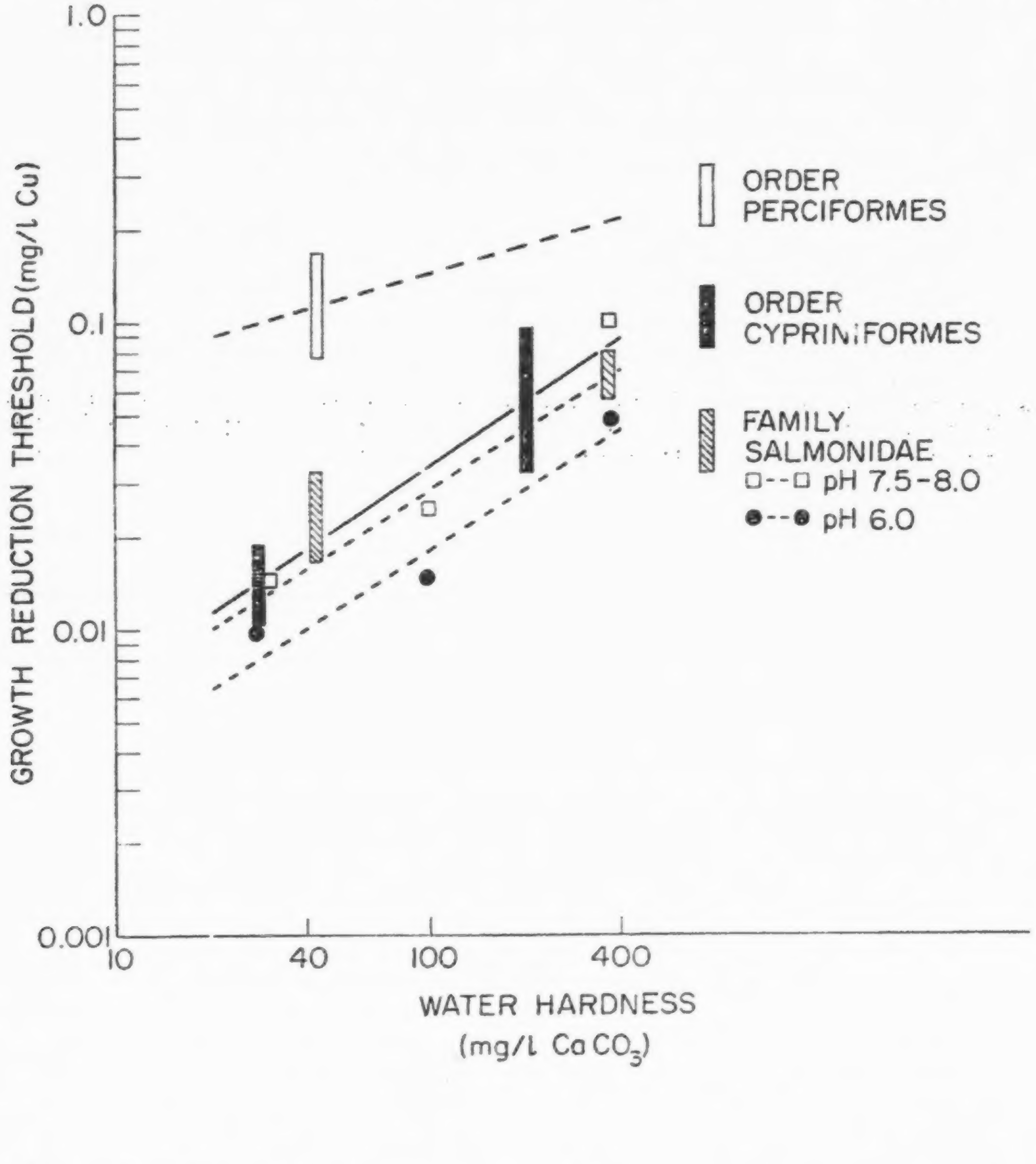

Figure 6: Interorder comparison of threshold for growth reduction of freshwater fish exposed to copper with respect to water hardness.

Due to the paucity of data, the relationships between sublethal tolerance and water hardness are, as yet, of limited predictive capability.

CONCLUSIONS

As a first approximation, the results of acute toxicity tests in which freshwater fish were exposed to copper solutions are at least partially delineated by quantifying the interrelation of water hardness and LC50. Unique equations were developed for each of the following taxonomic groups - the order *perciformes*, the order *cypriniformes*, the family *salmonidae*, and the genus *Oncorhynchus*. At the sublethal level of exposure to copper, similar relationships may exist between water hardness and threshold concentrations resulting in growth reduction.

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ABSTRACT

Vanadium (+5) was found to be moderate in toxicity among the metals. The 168 h median lethal concentration (LC50) of total dissolved vanadium (+5) varied from 2.02 mg/l in soft neutral water to 5.59 mg/l in hard acid water. Reported lethal levels for zinc to trout are slightly lower but similar.

In the tests, total hardness ranged from 30-360 mg/l as $CaCO_3$ and pH from 5.5 to 8.8 The 3-dimensional response surface was saddle-shaped. An increase in hardness usually made vanadium less toxic, while for all three hardnesses highest toxicity was at pH 7.7 and lowest toxicity at pH 5.5. A good prediction of LC50 ($R^2=0.98$) based on total dissolved vanadium for the full ranges of pH and hardness examined was given by the equation:

$$\text{LC50} = 4.8680784 + .1503267\ H - .927559\ pH - .0329045\ HpH$$

$$(\text{mg/l}\ V^{+5}) \qquad - .000089194\ H^2 + .054432\ pH^2 + .002204$$

H = total hardness, mg/l as $CaCO_3$
pH = - log hydrogen ion concentration

Vanadate ion speciation was determined by computer. All predicted ions were in the form of oxy-anions. Ionic composition of the test-water was dependent on the stability constants of the ions, the measured total metal concentration, and the pH. At a constant vanadium concentration, pH dictated the degree of protonation of the various ion species while at a constant pH, vanadium concentration affected ionic polymerization via the mass action laws.

Total ion concentration equalled the measured dissolved vanadium concentration only at high pH. Here, the predicted ion species contained one vanadium atom per ion. Two ions apparently contributed to the toxic response at alkaline pH. Of these, H_2VO_4- appeared more toxic than the HVO_4^{-2} ion. At neutral pH the ion H_2VO_4- was also definitely toxic as 90% of the ions at pH 6.6 and 7.7 were in this form. At low pH the picture was not so simple. The total ion concentration represented only a fraction (as low as 14%) of the total dissolved metal concentration due to the presence of polymeric ions (eg. $HV_{10}O_{28}^{-5}$). The degree to which the reduced toxicity at pH 5.5 was due to a differential in toxicity of the ions or due to the fewer number of ions in solution is not known. The inverse relationship between toxicity of vanadium to rainbow trout and hardness may have been due to the differential physiological acclimation of the trout and possibly cationic competitive inhibition. It is unlikely a result of the accompanied increase in alkalinity since vanadate chemistry is independent of the complexing components of alkalinity.

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Craig* G.R. and G.L. Beggs* 1979. Evaluation of fish loading rates in regulatory static bioassays. Proc. Fifth Annual Aquatic Toxicity Workshop, Hamilton, Ontraio, November 7-9, 1978. Fish. Mar. Serv. Tech. Rep. 862, pp. 145-160.

ABSTRACT

The validity of the fish loading rate criteria for regulatory bioassays of 1.0 litre $gram^{-1}$ day^{-1} was tested by conducting 96 hour static bioassays on single compounds and industrial wastes at five different rates (0.1, 0.5, 1.0, 2.0 and 3.0 ℓg^{-1} fish d^{-1}). All other conditions met the federal requirements for bioassay testing industrial effluents.

Rainbow trout fry were exposed to ammonium chloride, copper sulphate, thallous sulphate, a paper mill, a steel mill and a fertilizer manufacturing effluent. Results were expressed as LC50's and median survival times (MST's).

Ninety-six hour LC50's did not differ among loading rates while MST's varied according to the toxicant tested. These results indicated that regulatory tests completed at loading densities of 0.5 $\ell\ g^{-1}\ d^{-1}$ would provide equivalent 96-hour LC50's as tests carried out at 2.0-3.0 $\ell\ g^{-1}\ d^{-1}$ and would not affect the federal pass/fail criteria.

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EVALUATION OF FISH LOADING RATES IN REGULATORY STATIC BIOASSAYS

The fish loading rate in static toxicity tests has become increasingly important as these tests assume a greater role in the regulation of industrial waste discharges. Laboratories conducting regulatory tests can be faced with collecting and transporting large effluent volumes to satisfy loading criteria, particularly when test fish weight exceeds 1.0 g.

Where the availability of bioassay fish (rainbow trout in Canada) is limited due to import restrictions, laboratories must rely on local hatcheries. This places further demands on the bioassay test as fish are commonly in excess of 1.0 g for four months of the year.

Loading Rate History

The size of fish relative to the volume of bioassay sample has long been recognized as important to the result obtained (Table 1). Loading densities were initially expressed as grams per litre ($g\ \ell^{-1}$) with no time association. Doudoroff et al (1951) recommended a maximum of 2 g of fish ℓ^{-1} of sample (or $0.5\ \ell\ g^{-1}$) and that tests extend at least 48 hours but preferably 96 hours. They further recommended that loading density be decreased to $1\ g\ \ell^{-1}$ (or $1\ \ell\ g^{-1}$) if possible (Doudoroff 1951). Alabaster and Abram (1964) indicated that the Stevenage Water Research Center maintained loading rates at $0.5\ \ell\ g^{-1}d^{-1}$ for metals, ammonia and detergent tests but acknowledged that $1.0\ \ell\ g^{-1}d^{-1}$ was required to maintain concentrations of compounds lethal to fish within 24 hours (e.g. DDT and Rotenone). Studies on smaller harlequin fish, with higher respiration rates necessitated a density of $10.0\ \ell g^{-1}d^{-1}$. The APHA (1965) in developing fish bioassay standard methods adopted the loading density of $1.0\ \ell g^{-1}$ for both 48 and 96 hours. Test standardization through the APHA thereafter recognized the time factor and emphasis was placed on the volume of sample required per gram of fish rather than the converse. Sprague's (1969) review of Alabaster and Abram's work on fish respiration concluded that at least a $2.0\ \ell g^{-1}d^{-1}$ (preferably $3.0\ \ell\ g^{-1}d^{-1}$) density would be required for fish tests.

Canadian toxicity regulations were first published for the pulp and paper industry by Environment Canada (1971) under the Fisheries Act and the aspect of fish loading rates was avoided by requiring that the bioassay sample (65% effluent) be "replaced continuously at a constant rate". However, continuous flow tests are often impracticable. As an alternative, B.C. Research (1972) and Davis and Mason (1973) recommended that $1.0\ \ell\ g^{-1}$ (preferably $2.0\ \ell\ g^{-1}$) and $2.5\ \ell\ g^{-1}$ loading rates respectively be used in aerated static tests on pulp and paper wastes. Conclusions from these two studies were based on median survival time (MST) data for periods less than 12 hours.

The next five years of standard test development produced additional changes in fish loading criteria. Petroleum refinery guidelines (Environment Canada, 1974) required a 96 hour flow-through and 24 hour static bioassay with fish loadings of $1.4\ \ell\ g^{-1}d^{-1}$. The APHA (1975) increased fish loading criteria to $2.0\ \ell\ g^{-1}d^{-1}$ (preferably $3.0\ \ell\ g^{-1}d^{-1}$) for 96 hour static tests.

TABLE 1: Recommended fish loading rates for acute toxicity tests from 1951 to 1976.

Loading ($\ell\ g^{-1}d^{-1}$)	Remarks	Reference
0.13	96 hr. test; 0.25 preferable	Doudoroff et al 1951
0.25	48 hr. test; 0.5 preferable	" " "
0.5	metals, ammonia, detergents	Alabaster & Abrams 1964
1.0	DDT, Rotenone; 24 hr. toxicity	" " "
10.0	harlequin fish	" " "
0.5	1.0 over 48 hrs.	APHA 1965
0.25	1.0 over 96 hrs.	" "
2.0-3.0	from fish respiration data	Sprague 1969
1.0	2.0 preferable	B.C. Research 1972
2.5	12 hr. exposure	Davis & Mason 1973
2.0	3.0 preferable	APHA 1975
1.0	96 hr. acute test any type	Environment Canada 1976

Table 2: Dechlorinated Dilution Water Profile from Ministry of the Environment Rexdale Laboratory.

Parameter	N	$\bar{x}$ (mg l^{-1}) ± SD
Total Residual Chlorine	14	0.017 ± 0.006
Alkalinity ($CaCO_4$)	4	87.25 ± 0.96
Hardness ($CaCO_4$)	4	132.50 ± 1.00
Ca	4	39.75 ± 0.50
Mg	4	8.0 ± 0.0
Na	4	13.50 ± 0.58
K	4	1.45 ± 0.04
SO	4	30.38 ± 1.03
Conductivity (μmhos)	4	323.75 ± 9.46
pH	4	7.55 ± 0.26

Guidelines for metal mine effluent toxicity (Environment Canada, 1975) also established a loading criteria of 2.0 ℓ $g^{-1}d^{-1}$ in the continuous flow test and extended the static test to 96 hours. Textile mill effluent guidelines adopted the same loading rates for both 96 hour continuous flow and static tests (Environment Canada, 1976).

Shortly thereafter it became evident that this increased loading criteria of 2 ℓ $g^{-1}d^{-1}$ imposed overwhelming logistic problems for both consulting and regulatory bioassay laboratories. Bioassay samples of 160, 400 and 800 ℓ per concentration would be required for ten 2,5 and 10 g fish respectively, to meet the required 2.0 ℓ $g^{-1}d^{-1}$ in the 96 hour test.

The Environmental Protection Service Toxicity Technical Committee met during the 1976 Third Aquatic Toxicity Workshop to discuss the difficulties of running a practical test with rainbow trout. It was decided that the best approach, practically and scientifically, was to support a loading density of 1.0 ℓ $g^{-1}d^{-1}$. This reduced the volume of bioassay sample per concentration to 80, 100 and 200 ℓ for ten 2, 5 and 10 g fish respectively, exposed for 96 hours. If five fish (the minimum allowed in the guidelines) were exposed to each concentration the volumes would be reduced by 50 percent.

Over the past 25 years fish loading criteria have changed based primarily on data generated from 24 hour tests of median survival time and on fish physiological requirements for 24 hour tests. The emphasis is now on the 96 hour LC50 and it was felt that fish loading criteria should be evaluated using this response. This study was designed to determine whether 96 hour LC50's for rainbow trout exposed to single compounds and complex industrial wastes would vary with fish loading densities from 0.1 to 3.0 ℓ $g^{-1}d^{-1}$.

METHODS

Test Procedures

Rainbow trout (Salmo gairdneri Richardson) from Goossen's Trout Farm (Otterville, Ontario) were held at the hatchery water temperature (9° - 10°C) and raised to 15°C at 1°C per day then held at the test temperature for at least 7 days prior to testing. Stock mortalities for any batch did not exceed 1% for 7 days prior to testing.

Static bioassays conducted in dechlorinated Toronto tap water (Table 2) were aerated at 5 - 7.5 cc $\ell^{-1}min^{-1}$ and temperatures were held at 15 ± 1°C.

Mortalities were recorded at log intervals: 0, .3, .6, 1, 2.5, 5, 10, 24, 48, (72), 96 hours. Thallium observations were extended to 10 days with mortalities recorded every 24 hours after the first 96 hours. Control fish were weighed and measured at the end of each test.

Nominal fish loadings of 0.1, 0.5, 1.0, 2.0 and 3.0 ℓ $g^{-1}d^{-1}$ were established for each toxicant and each loading series incorporated 5 concentrations chosen to bracket the expected LC50 as determined by previous tests. One control was provided for each series. Ten fish were exposed to each concentration except where fish size required replicate concentrations of 5 fish per concentration.

Ninety-six hour LC50's and 95% confidence limits were calculated by probit analysis (Finney 1971). When no partial mortalities were observed a geometric mean was used to estimate the LC50 and confidence intervals were represented by the two test concentrations bracketing this value (Stephen 1977). Actual fish loadings were plotted against LC50's and individual differences were tested using the standard error of the mean (Sprague 1977). The LC50's were also tested by analysis of variance to check for differences with density among all toxicants. LC50 values from the 2 and 3 $\ell\ g^{-1}d^{-1}$ densities were treated as 2.5 $\ell\ g^{-1}d^{-1}$ to improve matrix design (only one of these densities was tested for each loading series).

Toxicants

Three toxicants were chosen for their different modes of biological action on fish. Analytical grade reagents were used to prepare all test concentrations.

Ammonia (NH_4Cl) was chosen for its action as a natural metabolite which reduces oxygen carrying capacity of blood and increases the metabolic rate in fish (Smart 1978). The concentration of unionized ammonia was calculated from NH_3-N values transformed according to Trussell (1972). Two different batches of fish from the same hatchery stock were exposed to ammonia one month apart.

Copper ($Cu\ SO_4 \cdot 5\ H_2O$) was used due to its capability of detaching the epithelial layer from gill lamellae and the inhibitory effect on oxidative enzyme activity in the gill (Bilinski and Jonas 1973).

Thallium (Tl_2SO_4) was chosen for its apparent action on ATPase and its ability to substitute monovalent cations in enzymatic reactions (Zitko 1975).

Three complex industrial wastes were selected on the basis of their distinctly different profiles.

1) Cyanamid of Canada (Welland) effluent from the ammonia and urea plant producing fertilizer and cattle feed supplements, was selected as representative of manufacturing process wastes characterized by high ammonia levels (>100 mg/l). Ammonia was measured in the bioassay samples, however, the effluent profile was drawn from historical data (Table 3).

2) Waste from the Algoma Steel Company (Sault Ste Marie) was tested as representative of the cyanide, ammonia, phenols and metals composition common to the steel industry (Table 4).

3) The final effluent from the Spruce Falls Power and Paper Company (Kapuskasing) thermal mechanical refining process was selected for the resin and fatty acids indicative of pulp and paper wastes (Table 5).

Table 3: Effluent Profile from Cyanamid Ammonia and Urea Plant (based on historical data).

Parameter (n = 11)	mg l^{-1}
Ammonia	660 ± 310
Urea	≃436 (R = .661)
pH	9.23 ± 0.29
Total Solids	2474 ± 2975

Table 4: Effluent Profile from Algoma Steel Thickener Overflow

Parameter	mg l^{-1}
Ammonia	1.7
Phenol	.005
Cyanide	3.6
Iron	.05
Copper	.02
Zinc	.45
Manganese	.03
Total Solids	170
pH	8.5
Conductance (μhmos)	260

Table 5: Final Effluent Profile from Spruce Falls Power and Paper Company. Thermal Mechanical Refining Process.

Resin Acids	mg l^{-1}
Isopimaric	10.1
Pimaric	2.4
Sandaraco-Pimaric	4.71
Levo-Pimaric	4.41
Abietic	85.91
Dehydroabietic	16.38
Neo-Abietic	55.15
Fatty Acids	
Oleic	.52
Linoleic	2.10
Benzoic	7.51

Ammonia concentrations were measured according to the Standard Methods (APHA 1975) technique. Metals were measured by AAS while resin and fatty acids were measured by GC (Ontario Ministry of the Environment 1974). Measured concentrations of ammonia, copper and thallium were used to calculate LC50's.

RESULTS AND DISCUSSION

Chemicals

Ammonia results for runs A and B appear to differ with respect to individual tests at the 0.1 and 0.5 $\ell\ g^{-1}d^{-1}$ loading rate. This may be due to size-related differences in resistance between the two groups. Group A was 1.4 g compared with a mean weight of 1.0 g in group B. However, highest and lowest densities were not significantly different in both cases (Fig. 1). Based on the average test pH of 7.5 and 15°C temperature the average 96 hour LC50 of 0.4 ± 0.2 mg ℓ^{-1} of un-ionized ammonia agrees well with the reported 24 hour LC50 of 0.5 (Ball 1967) and the 48 hour LC50 of 0.48 mg ℓ^{-1} un-ionized ammonia (Herbert and Shurben 1964) for rainbow trout.

Copper lethality varied little with the densities tested (Fig. 1). The mean 96 hour LC50 was 0.12 ± 0.01 mg ℓ^{-1} which compared well with Howarth and Sprague's (1978) projected 96 hour LC50 of 0.14 mg ℓ^{-1} for 10 g rainbow trout (hardness 132 mg ℓ^{-1} as Ca CO_3; pH 7.8).

Thallium 240 hour LC50's were also unaffected by changes in fish loading (Fig. 1). The mean 240 hour LC50 of 1.5 ± 0.5 mg ℓ^{-1} fell between the lethal concentrations of 10-15 mg 1^{-1} observed for rainbow trout (Nehring 1962) and the 240 hr. LC50 of 0.24 mg ℓ^{-1} for Atlantic salmon (Zitko 1975).

Previously reported loading evaluations support the above results. The 96 hour LC50 values for dodecyl sodium sulphate (DSS) were essentially the same for loading rates between .25 and 2.5 $\ell\ g^{-1}d^{-1}$ (Pessah et al. 1975). Tests were completed on different fish stocks at different times (Fig. 2). Similarily, 96 hour LC50's of rainbow trout exposed to sodium pentachlorophenate under static unaerated conditions differed only when loadings were less than 0.5 $\ell\ g^{-1}d^{-1}$ (E.V.S. Consultants 1977).

Industrial Wastes

Cyanamid 96 hour LC50's were similar for all loadings tested (Fig. 3). The measured ammonia concentration in the effluent was about 6.9 mg ℓ^{-1} un-ionized ammonia. The average LC50 of 3.2% waste contained an equivalent of 0.2 mg ℓ^{-1} un-ionized ammonia which is within the range observed for the ammonium chloride exposures.

LC50's of rainbow trout exposed to the Algoma Steel thickener discharge were also comparable among the fish loadings tested, with a mean 96 hour LC50 of 3.6% (Fig. 3). Dilution to 3.6% waste would reduce cyanide to 0.12 mg ℓ^{-1}, ammonia to 0.06 mg ℓ^{-1} and zinc to 0.02 mg ℓ^{-1}. Cyanide has been reported to be acutely toxic to rainbow trout at 0.09 mg 1^{-1} as HCN (Doudoroff 1976). Ammonia and zinc would not appear to

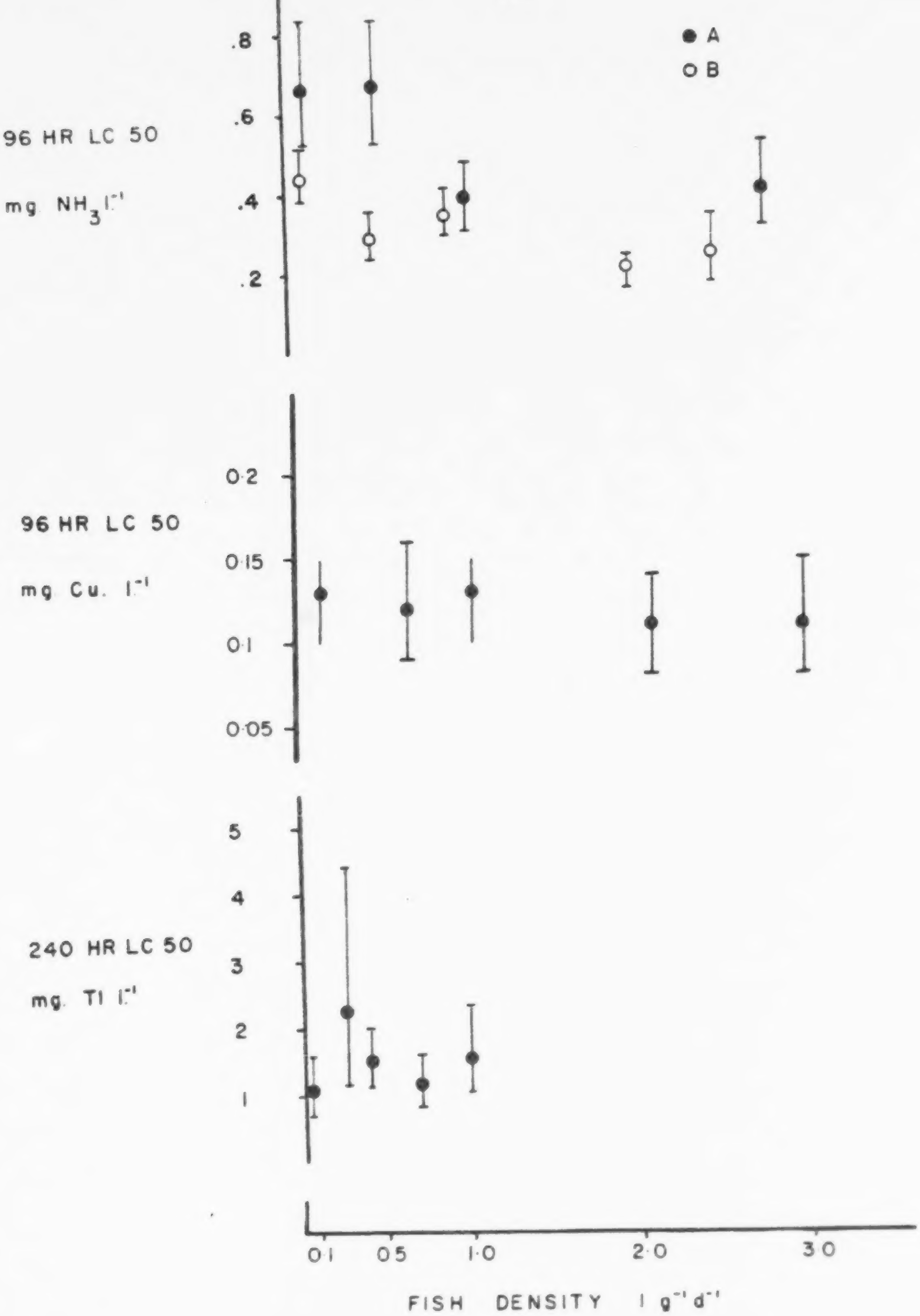

Figure 1: Static 96-hr. and 24-hr. LC$_{50}$'s for rainbow trout exposed to ammonia (un-ionized) copper (total) and thallium (total) at actual loading densities from 0.1 to 3.0 ℓ g^{-1}d^{-1}. Confidence limits (95%) are represented with tailed bars while lethal ranges (0-100% mortality) have straight bars.

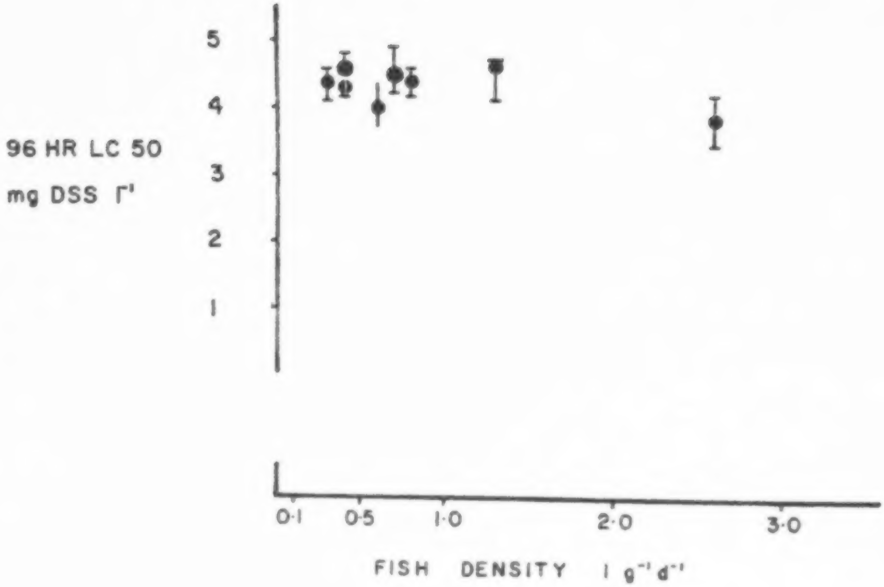

Figure 2: Static 96-hr. LC_{50}'s for rainbow trout exposed to dodecylsodium sulphate (DSS) at actual loading densities from 0.1 to 2.5 $\ell\ g^{-1}d^{-1}$. Confidence limits (95%) are represented with tailed bars while lethal ranges (0-100% mortality) have straight bars. Data from Pessah, Wells and Schneider, 1975.

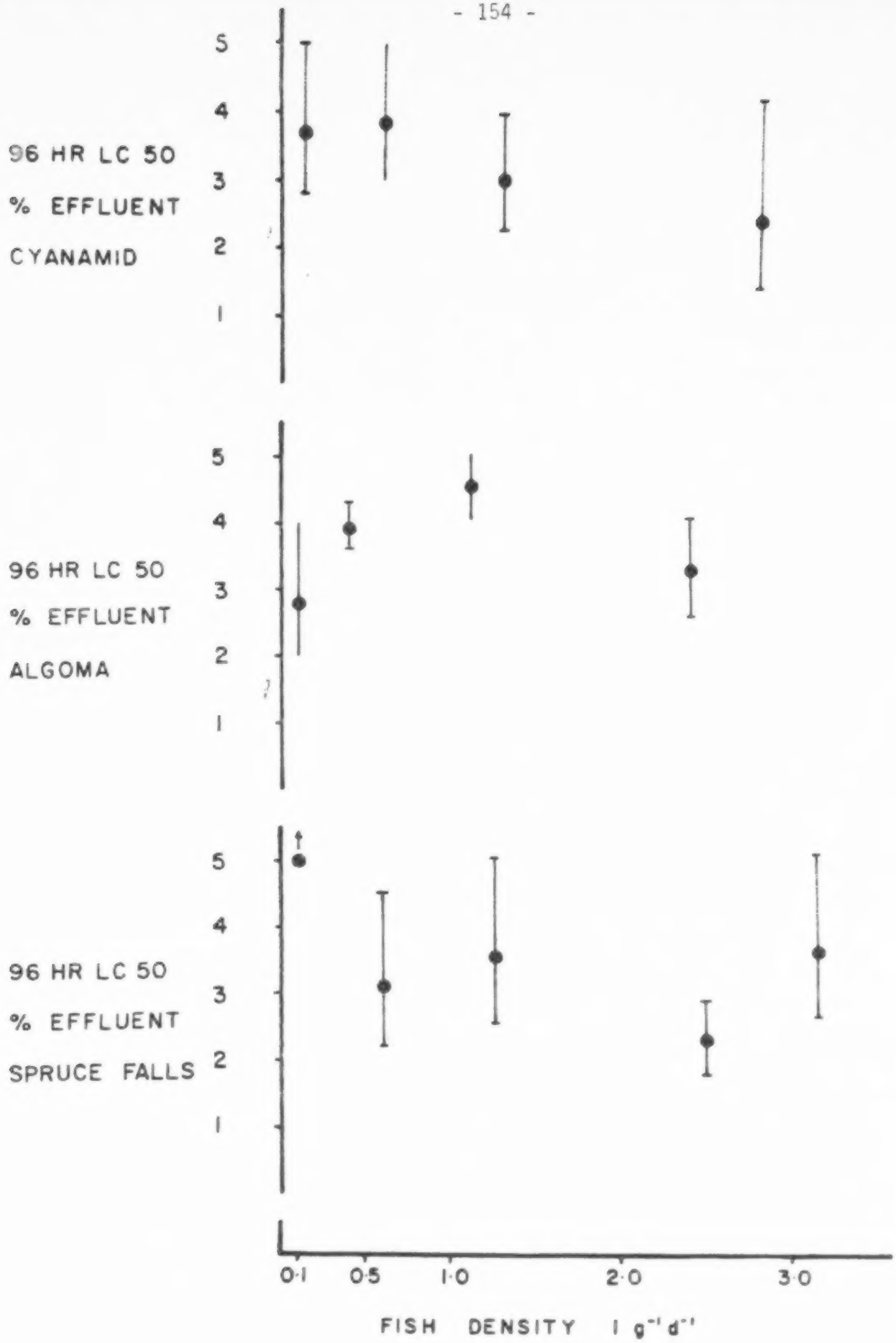

Figure 3: Static 96-hr. LC_{50}'s for rainbow trout exposed to industrial effluents from fertilizer manufacturing (Cyanamid), a steel mill (Stelco) and pulp and paper mill (Spruce Falls), at actual loading densities from 0.1 to 3.0 $\ell\ g^{-1}d^{-1}$. Confidence limits (95%) are represented by

contribute significantly to lethality of this waste at dilutions of 3.6%. [96 hour LC50 of zinc for rainbow trout is 1 - 2 mg ℓ^{-1} (EPA 1976)]. The presence of zinc, copper and iron in the thickener discharge could result in cyanide complexes and consequently reduce the toxicity (Doudoroff 1976).

Tests with Spruce Falls effluent produced equivalent 96 hour LC50's at all loadings with the exception of the 0.1 $\ell\ g^{-1}d^{-1}$ density. The highest concentration tested (5%) at this density produced 10% mortality (Fig. 3).

Analysis of variance for chemical and effluent LC50's indicated significant homogeneity ($p > 0.05$) across the loading gradients tested. The 0.1 $\ell\ g^{-1}d^{-1}$ density, however, violated the minimum depth requirement of 15 cm for bioassay samples and would consequently be eliminated from regulatory tests. Interpretation of the remaining 96 hour LC50 data indicates that tests at a fish loading rate of 0.5 $\ell\ g^{-1}d^{-1}$ yield LC50's representative of the 2 - 3 $\ell\ g^{-1}d^{-1}$ loading rates recommended in the past.

MST and Loading Rates

The relationship between median survival time (MST) and fish loading rate for selected concentrations of ammonia, copper, Cyanamid, Algoma, and Spruce Falls samples is depicted in Figure 4. No differences in MST's were recorded for Algoma and Cyanamid samples. Ammonia and copper MST's increased with loadings less than 0.5 $\ell\ g^{-1}d^{-1}$. The MST's for Spruce Falls, however, increased at loadings less than 1.3 $\ell\ g^{-1}d^{-1}$ (Fig. 4) supporting the data of Davis and Mason (1972) for bleached kraft mill effluent. In another study, rainbow trout exposed to neutralized unbleached kraft white water under static aerated conditions had a similar MST response pattern at fish loading rates from 0.2 - 2.5 $\ell\ g^{-1}$ (B.C. Research 1972). In the latter study additional MST tests indicated that, with aeration, surface area to volume relationships of the bioassay sample did reduce toxicity when ratios were large. The authors preferred, therefore, to remain with the APHA recommendation of 1.0 $\ell\ g^{-1}d^{-1}$ due to the limited exposure periods studied (less than 8 hours) and the limited data available for pulp and paper mill effluents (B.C. Research 1972).

Median survival times recorded for rainbow trout exposed to thallium gradually decreased with a reduction in loading rate in both the 1.5 and 2.5 mg ℓ^{-1} test concentrations, illustrating the unusual response of fish to this toxicant (Fig. 5).

Median survival time data generated from tests with ammonia, copper, Algoma and Cyanamid effluents indicate a fish loading rate of $\geq$ 0.5 $\ell\ g^{-1}d^{-1}$ is recommended. However, data from Spruce Falls suggests a loading of $\geq$ 1.0 $\ell\ g^{-1}d^{-1}$ may be preferable. Median survival time trends for thallium indicate loadings less than 0.4 $\ell\ g^{-1}d^{-1}$ would provide the most sensitive test, which is logically inconsistent with accepted dose-response relationships. However, as MST data are often developed from short-term tests using relatively high concentrations of toxicant it is unreasonable to develop loading criteria for longer term 96 hour tests from these results. Additionally MST response to loading rates can differ from that of the LC50. As effluent regulations are currently based on the 96 hr. LC50 it is logical to base loading rate recommendations on this same response.

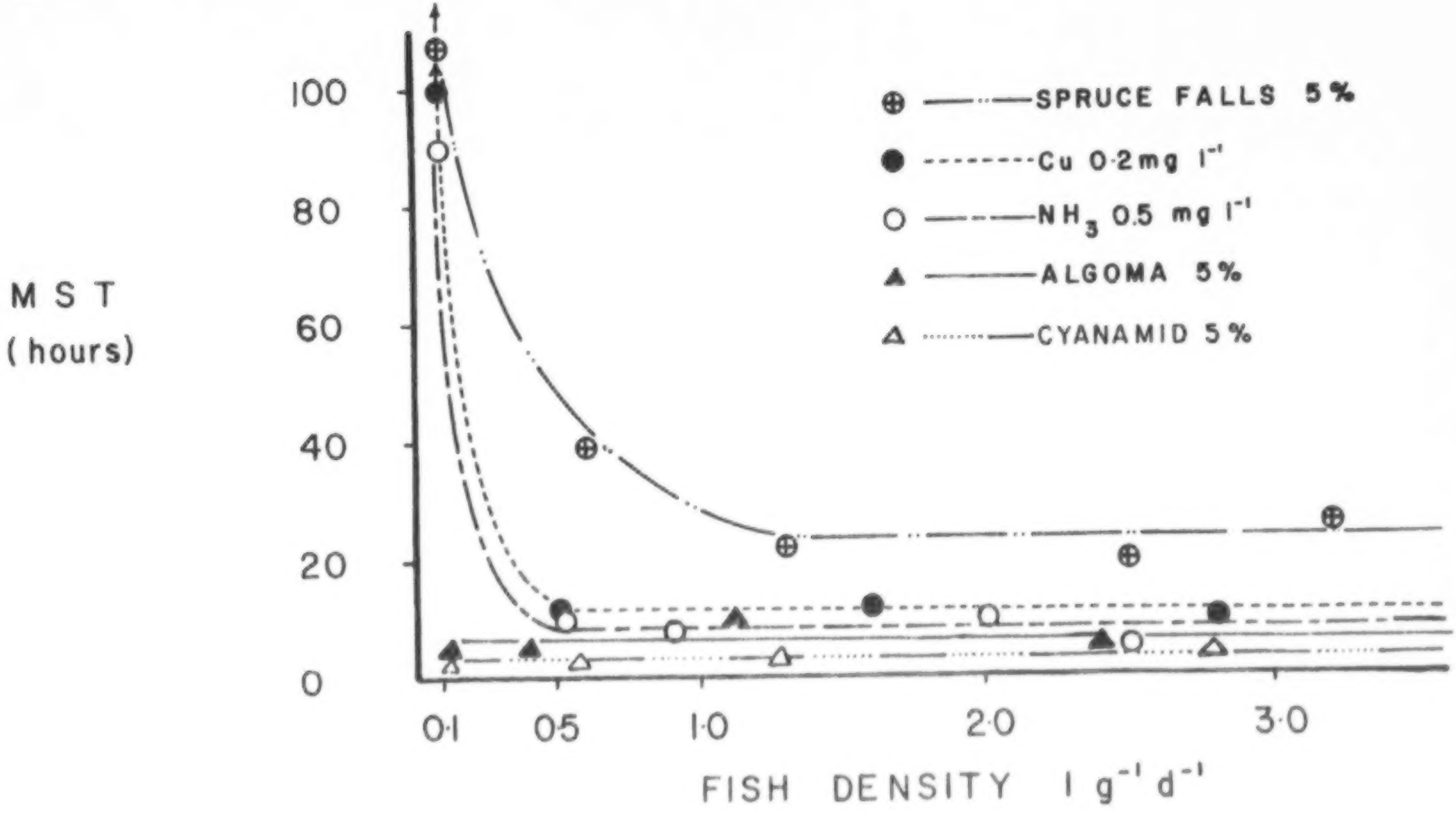

Figure 4: Median survival times (MST) for rainbow trout exposed, under different loading densities, to single ammonia, copper and three industrial effluent concentrations.

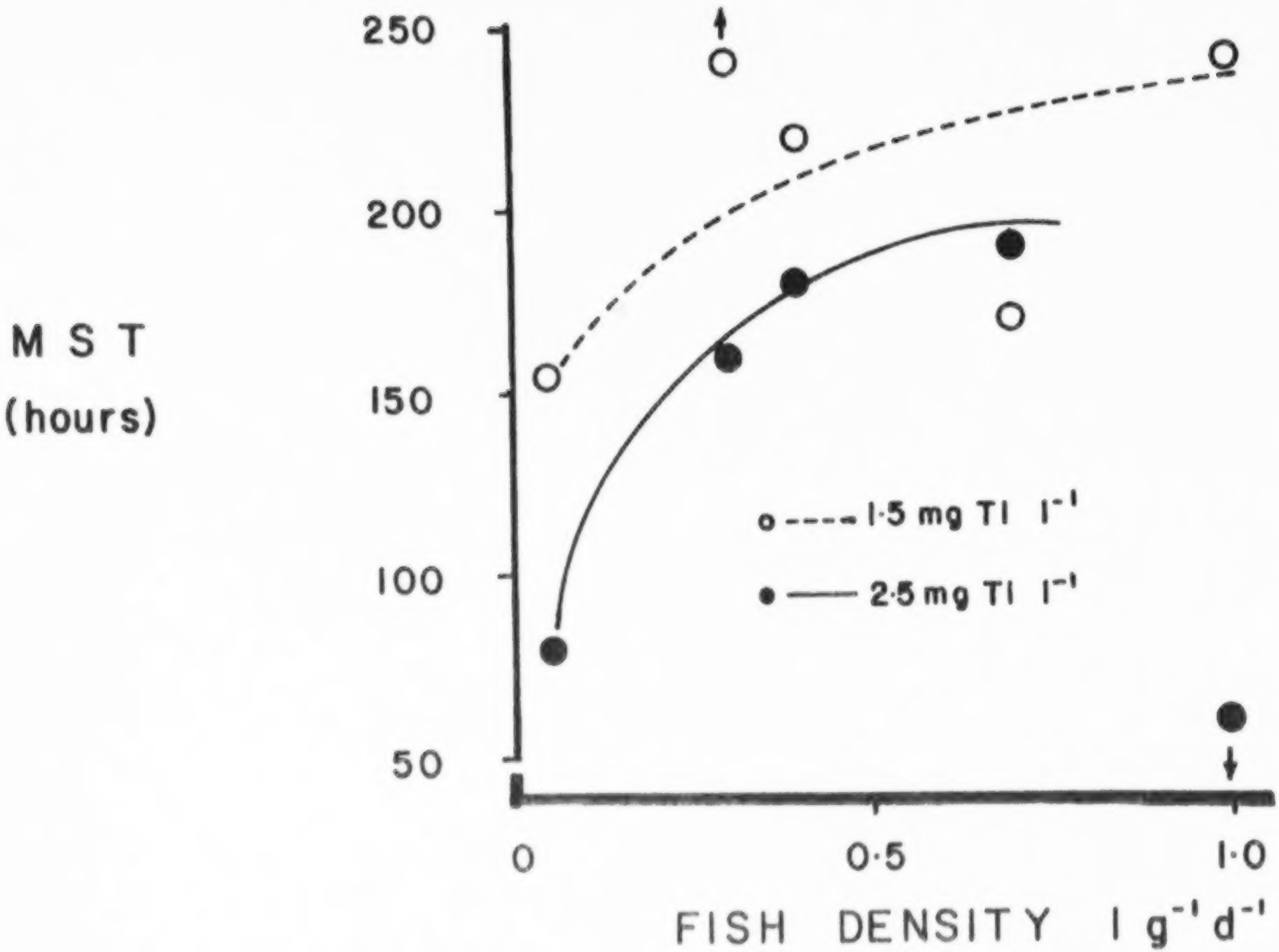

Figure 5: Median survival times (MST) for rainbow trout exposed, under different loading densities, to two concentrations of thallium.

Recommendations

Fish loading requirements for regulatory aerated 96 hour static bioassays may be reduced to $0.5\ \ell\ g^{-1}d^{-1}$ without significantly affecting the LC50. Many aspects of bioassay testing would be more manageable with the $0.5\ \ell\ g^{-1}d^{-1}$ density criteria i.e. smaller effluent volumes would be required, larger fish could be tested, less laboratory space would be needed, probably resulting in reduced resource costs.

Extensions of this fish loading recommendation to other test types, particularly those in which oxygen concentrations may decrease, should be applied with caution. As outlined previously, bioassay test criteria should only be developed from methods evaluations conducted under the required test conditions.

Acknowledgements

The author's appreciation is extended to H. Clark, B. Flock and G. O'Hara for their assistance with this project.

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ABSTRACT ONLY

Several statistical models are considered for studying the relationship between time, concentration, and mortality and the subsequent estimation of 'threshold' in acute toxicity studies. Bliss's model (1940) is formalized and estimated by maximum likelihood. Box & Cox transformations are suggested as aids in choosing the appropriate time metameter. A test is given for testing the appropriateness of the selected metameter. Statistical models corresponding to the kinetic models discussed by Rubin and Elmaraghy (1977) are presented. The proposed models are evaluated on several data sets. Particular attention is given to the effect of model selection on the precision of the estimated threshold and to the selection of concentrations for precise estimation of threshold. Finally, methods are discussed for examining the relationship between LT50 and concentration and also for determining the distribution of survival times of the organism being studied.

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**Department of Health and Welfare, Food Statistics and Operational Planning, Ottawa, Ontario.

Dixon*, D.G. 1979. Acclimation to toxicants by rainbow trout and its potential use in predicting safe levels. Proc. Fifth Annual Aquatic Toxicity Workshop, Hamilton, Ontario, Nov. 7-9, 1978. Fish. Mar. Serv. Tech. Rep. 862, pp. 162-166.

ABSTRACT

A series of experiments were undertaken to investigate the effect of pre-exposure or acclimation of rainbow trout to sublethal levels of copper on their subsequent resistance to lethal copper levels.

Following a six-week period of adaptation to the water conditions maintained during the experiments, fish were exposed for 21 days to a sublethal level of copper. Three experiments were carried out involving pre-exposure to 33, 95, 140 ugl^{-1} copper. Control fish were maintained under identical conditions in the absence of toxicant. On days 0, 7, 14 and 21 of the sublethal exposure period, two 144-hour lethality bioassays were initiated, one using exposed fish and one using control fish. Each bioassay utilized 6 tanks, 5 concentrations of copper and one control, with 20 fish per tank. These tests were carried out at 15°C in water having a pH of 7.8 - 8.0, a total hardness of 360 - 380 mg l^{-1} as $CaCO_3$, and a dissolved oxygen level of not less than 90% saturation. The 1.5 - 3.5 g fish were fed Silver Cup ration at a rate of 2.5% of their wet body weight per day during the sublethal exposure period. They were not fed during the lethality bioassays.

Acclimation resulted in changes in the LC50 of the experimental fish relative to controls. Those fish pre-exposed to 95 and 140 ugl^{-1} Cu demonstrated significantly increased 144-hr Cu LC50s. The degree of induced copper resistance was dependent on both the concentration and duration of the pre-exposure. Conversely, fish acclimated to 33 ugl^{-1} Cu demonstrated a reduced copper resistance. These fish consistently demonstrated copper LC50s lower than those obtained with control organisms. A threshold level of acclimation was therefore evident, above which the test organisms were more resistant to copper, and below which they were less resistant (Figure 1).

This graded and reversing response to acclimation could potentially be used for estimating a safe level for copper. The level of pre-exposure at which the acclimated fish first demonstrate an increased resistance to copper would represent a threshold of sublethal toxicity, a level below which no detrimental effects would be expected to occur. The ratio of this threshold value to the LC50 of the control fish would represent an application factor for copper. Using this method and the data reported in Figure 1, an application factor for copper of 0.11 was obtained, a value which compares favourably with the copper application factor of 0.1 widely accepted in the literature.

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The protocol outlined provides a rapid and inexpensive method for detemining safe levels on the basis of bioassay technology, without the need to carry out costly and complex sublethal studies. These techniques could be applied to any toxicant or effluent which demonstrated an acclimation dose-response relationship.

Experiments utilizing the same experimental design and conditions outlined for copper were carried out with cyanide (Figure 2) and arsenic (Figure 3). Although the acclimation responses for these two environmental contaminants differ from that of copper, in both instances significant alteration of the lethal response resulted from pre-exposure. Acclimation dose-response curves and application factor estimates could, therefore, be developed for both of these toxicants.

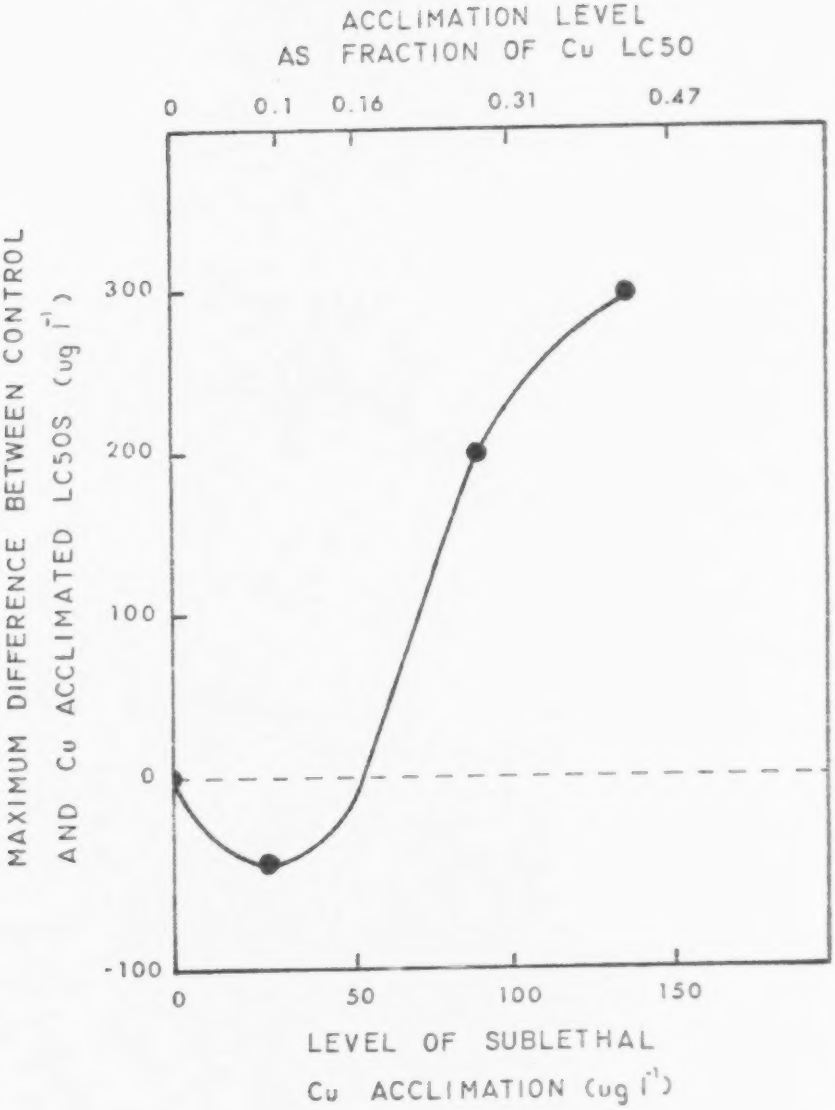

Figure 1. The relationship between the level of sublethal copper exposure and the difference between the control and experimental LC50s (acclimated LC50 - control LC50) after 21 days of copper pre-exposure. The pre-exposure level is expressed both as concentration of copper (lower abscissa) and as a fraction of the mean control LC50 (315 $\mu g l^{-1}$) (upper abscissa).

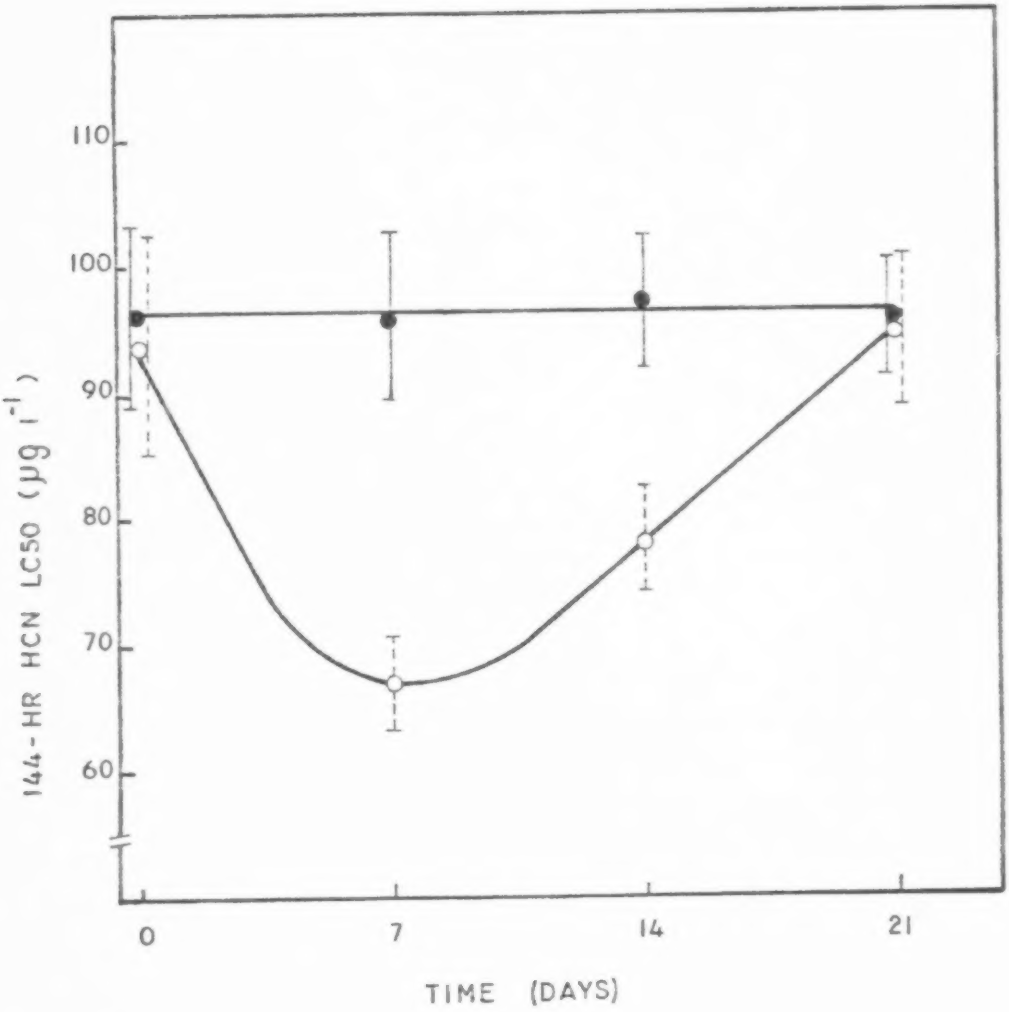

Figure 2. The relationship between the period of exposure to a sublethal level of HCN (33.8 μgl^{-1}) and the 144-hour LC50 of HCN for rainbow trout. Each point represents one of the 144-hour LC50s with its 95% confidence interval. Solid points and solid confidence intervals represent control fish, while open points and broken confidence intervals indicate sublethally exposed (acclimated) fish.

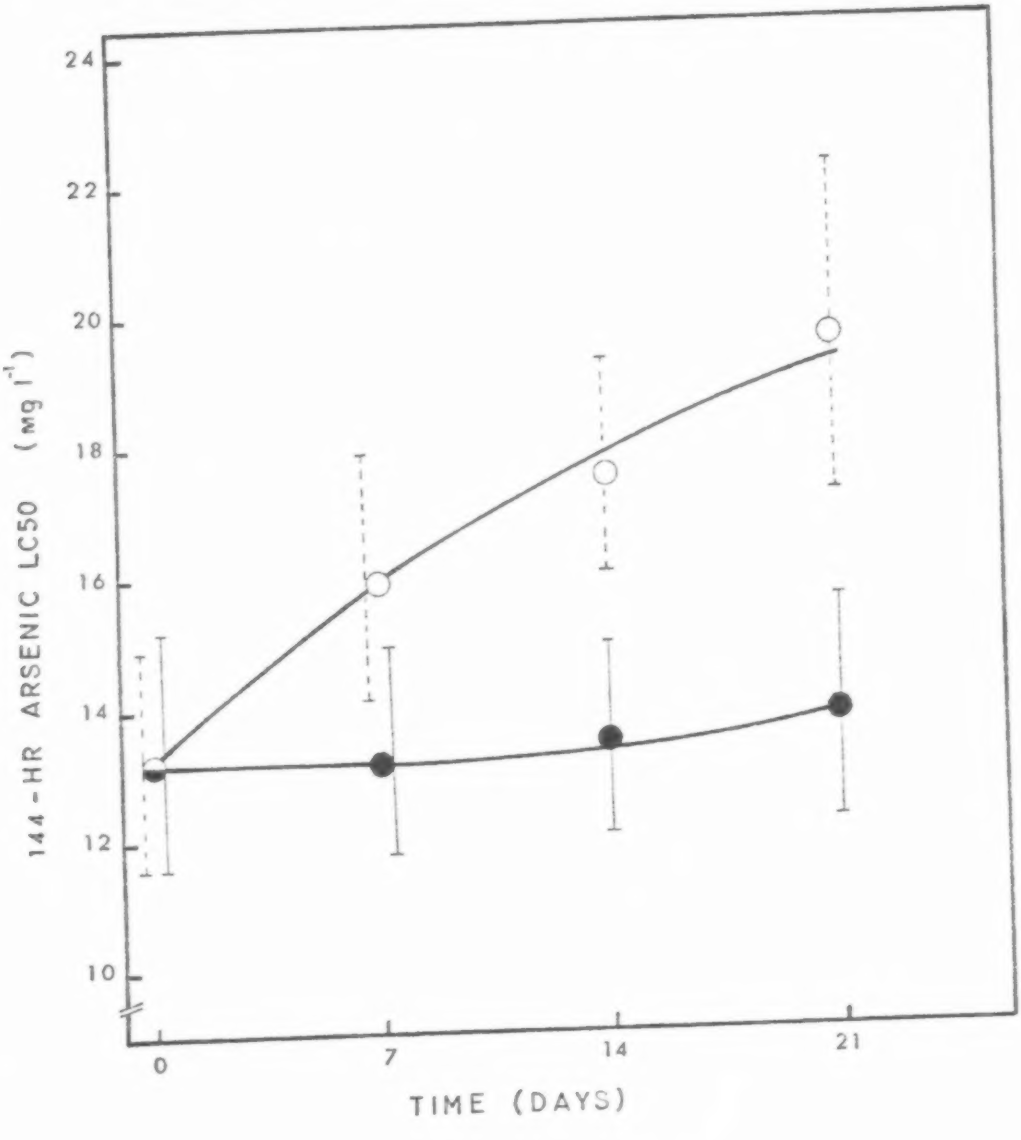

Figure 3. The relationship between the period of exposure to a sublethal level of arsenic (2.96 mg l^{-1}) and the 144-hour incipient arsenic LC50 with its 95% confidence interval. Solid points and solid confidence intervals represent control fish, while open points and broken confidence intervals indicate sublethally exposed (acclimated) fish.

Hammond*, B.R., and J.R. Bishop**, Jr. 1979. The physiological evaluation of ozone and chlorine toxicity on channel catfish, _Ictalurus punctatus_. Proc. Fifth Annual Aquatic Toxicity Workshop, Hamilton, Ontario. Nov. 7-9, 1978. Fish. Mar. Serv. Tech. Rep. 862, pp. 167-182.

ABSTRACT

The 96 hr LC_{50} for ozone and chlorine exposure on fingerling channel catfish, _Ictalurus punctatus_, as determined from flow through bioassays was 0.03 mg/l ozone and 0.07 mg/l chlorine. Short term chlorine ozone exposures had little effect on kidney function. The gills rapidly removed chlorine from solution. Gill Na uptake was drastically impaired by both ozone and chlorine. Blood pressure and to some extent heart rate were the most sensitive physiological parameters for low level (96 hr LC_{50}) chlorine exposure. At exposures of 1/2 the 96 hr LC_{50} no changes in response were observed. Low lethal levels of ozone produced no circulatory response. Thus it can be concluded that on exposure to a chlorine concentration of 0.007 mg/l (1/10 of 96 hr LC_{50}) fish should show no physiological disfunction. Our 96 hr LC_{50} of 0.03 mg/l ozone is at the limit of analysis and could not be detected in the presence of fish.

Key words: Toxicology, chlorine, ozone, freshwater, pollution, physiology, blood pressure, kidney, gills, fish, catfish.

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INTRODUCTION

With the present emphasis on the maximum utilization of water resources and the concern for the protection of public health, the use of ozone instead of chlorine in disinfection has many environmental advantages. Ozone unlike chlorine is incapable of producing a residual with extended action. This residual effect of chlorine was reported to have caused species shifts in a Maryland river (Tsai, 1968). Also, Arthur and Eaton (1971) showed that the reproduction of fathead minnows was drastically affected by exposure to sublethal concentrations of chlorine. Recent reviews by Brungs (1973,1976) suggests that continuous exposure to 0.003 mg/l or less of residual chlorine would not harm most aquatic organisms, but continuous exposure to 0.01 mg/l would harm some species of fish at some life stages. In another review on chlorine by Becker and Thatcher (1973) no acceptable chlorine standard had been established.

For ozone much data is documented in the extensive bibliography entitled "Ozone Applications in Water Purification and Pollution Control". Although there is an abundance of information on the control of pathogenic organisms, there is a paucity of data on aquatic impact. The early work by Hubbs (1930) showed that ozone concentrations of 0.1 mg/l were lethal to fish and invertebrates. MacLean, et. al. (1973) found ozone resulted in retardation and abnormal egg development in American oysters (*Crassostrea virginica*). Although ozone is shown to be toxic to aquatic organisms it has been successfully used to control disease in incubating trout eggs (Benoit, and Matlin, 1966). However, its use is not widespread since over exposure results in death.

The present studies were designed to measure the 96 hr LC_{50} in channel catfish and to evaluate several physiological parameters in fish under chlorine and ozone stress.

Methods

Adult (0.25 -2.0 kg) and fingerling (6-10 cm) channel catfish, *Ictalurus punctatus* were collected and held at 22± 0.5°C in 550 l circulating tanks. The regime was 12 hours light and 12 hours dark. The fish were fed three times weekly. All fish were acclimated for two weeks before being used in experiments.

For collection of physiological data adult fish were anesthetized with "MS-222" and both the caudal vein and caudal artery (dorsal aorta) were cannulated with PE 50 tubing using a modified Seldinger technique (Hoar and Hickman, 1975). The bladder was catheterized with a heat shaped bilumen catheter. Following the operation the fish was placed in a clear plastic restraining box and allowed to acclimate for three days. Water was pumped through the box at 3-5 l per min. Several hours before an experiment the dorsal aortic cannual was connected to a pressure transducer and recording physiograph.

For sodium uptake experiments a fish was placed in a 10 l recirculating system. The system contained deionized water to enhance uptake. A known amount of NaCL labelled as ^{22}Na (Amersham/Searle) was added to the system and its disappearance from the water monitored.

Static bioassays (96 hr LC_{50}) were run on fingerling channel catfish using the procedure described in Standard Methods (13th Edition). Chlorine added as sodium hypochlorite was returned to the initial value every 24 hours. Through monitoring it was observed that chlorine was rapidly removed from the containers by respiring fish.

Using procedures similar to those described by Sprague (1972) and Standard Methods (13th Edition) continuous flow or flow through bioassays (96 hr LC_{50}) were run on fingerling channel catfish. Soldium hypochlorite was continually added to the inflow water by a calibrated syringe infusion pump, resulting in a constant chlorine concentration in the water. Ozone (generated from pure oxygen) was continually added by aeration at the inflow. Ozone concentrations were controlled by regulating the oxygen supply flowmeters. At the lower levels ozone concentrations in the water were extrapolated from known ozone concentrations and their oxygen flow rates.

For chlorine uptake experiments an adult fish was placed in a 40 l circulating tank. A known dose of sodium hypochlorite was introduced and the concentration of chlorine monitored through time until disappearance.

All ions were analysed by atomic absorption according to Standard Methods (13th Edition). Both chlorine and ozone were measured with a Wallace and Tiernan Amperometric Titrator using Wallace and Tiernan reagents as described in Standard Methods (13th Edition). The samples were analysed within minutes following collection.

RESULTS

Bioassay

The survival-mortality characteristics of fingerling channel catfish to chlorine and ozone were examined in three duplicate experiments (Figs. 1 and 2). For chlorine the flow through bioassay compared to the static bioassay results in a lower 96 hr LC_{50}, 0.07 mg/l chlorine vs. 0.45 mg/l chlorine. For ozone the flow through 96 hr LC50 is 0.03 mg/l. The concentrations at which 50% of the fish survived was read directly from the graphs. These concentrations represent the measured amounts of chlorine and ozone added to the inflow of the flow through system. Because of rapid disappearance chlorine and ozone could not be detected in the test containers at these low concentrations when fish were present. Thus the concentrations shown for the flow through systems are levels that would be representative at treatment outfalls.

Kidney Function

The results of the flow through experiments established a reference point for the physiological evaluations on adult catfish. Kidney functions (ion excretion, urine flow and glomerular filtration rate) were monitored before, during, and after exposure to chlorine and ozone. The concentrations added ranged from 0.36 to 1.8 mg/l for ozone and 0.2 to 0.4 mg/l for chlorine. Chlorine was added as a single dose and was measured in the system after 30 minutes. Toxic concentrations of ozone were continuously added for 12-24 hours. After carefully observing the kidney functions of catfish over a period of several days before and after exposure to chlorine and ozone, it is concluded that short term exposure does not significantly affect glomerular filtration rate, urine flow nor ion excretion.

Gill Chlorine Uptake

Chlorine is rapidly removed from solution in the presense of fish. This was deduced from the experimental data in Fig. 3. In these studies, a different concentration of chlorine (1.5 - 10 mg/l) was added to the 40 l circulating system for each fish. The average disappearance rate of chlorine for a respiring fish is relatively constant at 0.4 mg chlorine per min per kg of fish and is valid over a wide range of sizes (0.37 - 1.65 kg). The disappearance of chlorine from the system is only slightly less than the small amount removed when a fish with no opercular movements (anesthetized with nembutal via caudal cannula just prior to the chlorine addition) is in the holding box. The conclusion from these experimental data is that chlorine is rapidly removed by the gills of fish and reacts only slightly with the body surface and respiratory metabolites secreted by the gills. No urine is present because of catheterization.

Gill Sodium Transport

The influence of chlorine and ozone on the uptake of ^{22}Na by gills of catfish is shown in Figs. 4 and 5. Phase one of the experiment utilized the fish as its own control. A one day recovery period followed. The addition of 0.1 mg/l of chlorine at the start or the continuous addition of 0.1 mg/l ozone markedly reduced the ability of the gills to remove sodium from the aqueous solution. This reduction in uptake rate suggests an impairment of normal physiological function in the sodium transport system of the gill epithelium.

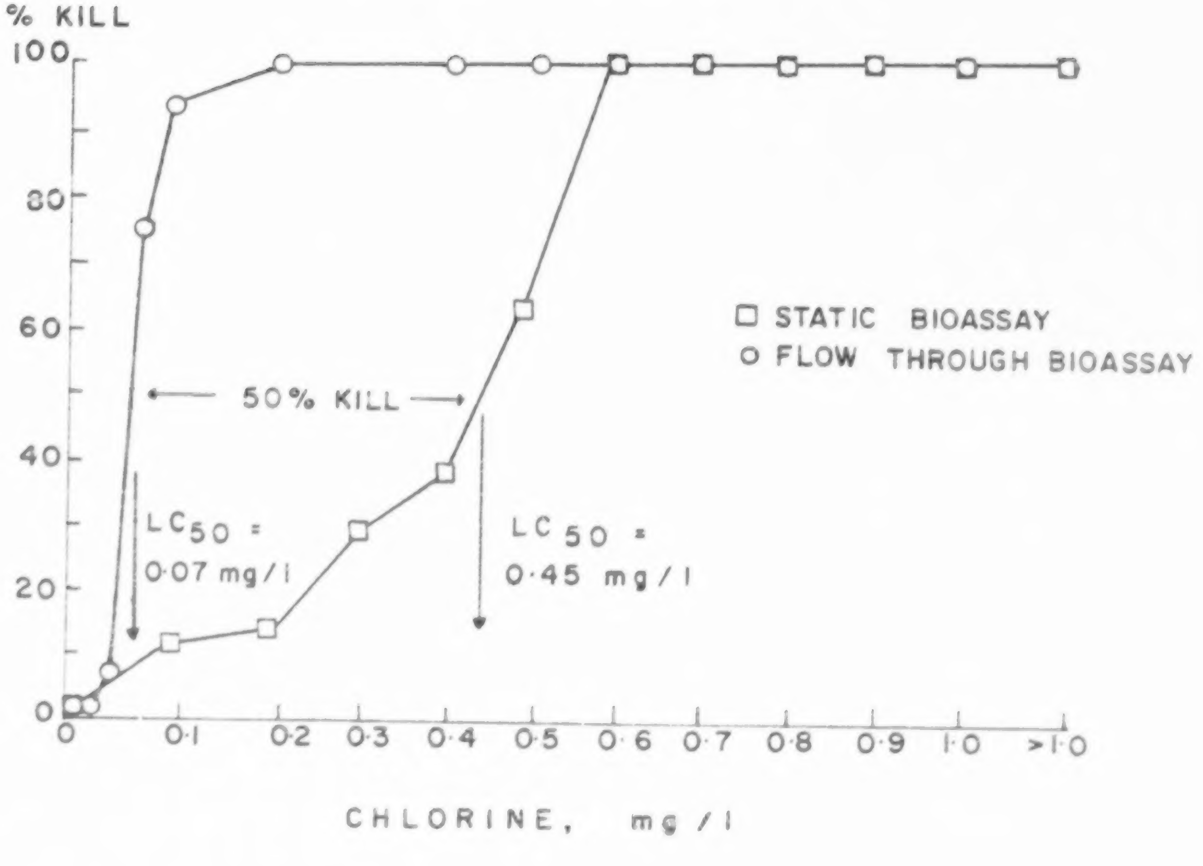

Figure 1 A comparison of survival-mortality characteristics of fingerling catfish to chlorine exposure in a static bioassay and in a flow through bioassay.

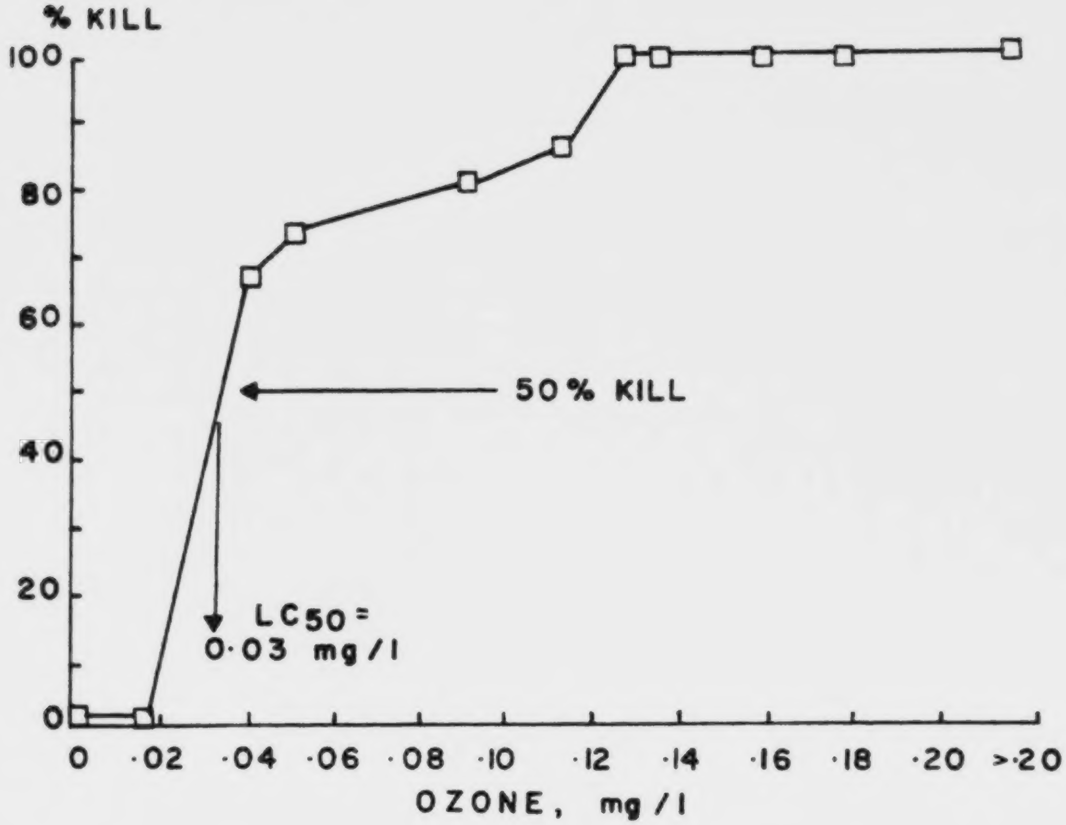

Figure 2 Survival-mortality characteristics of fingerling catfish to ozone exposure in a flow through bioassay.

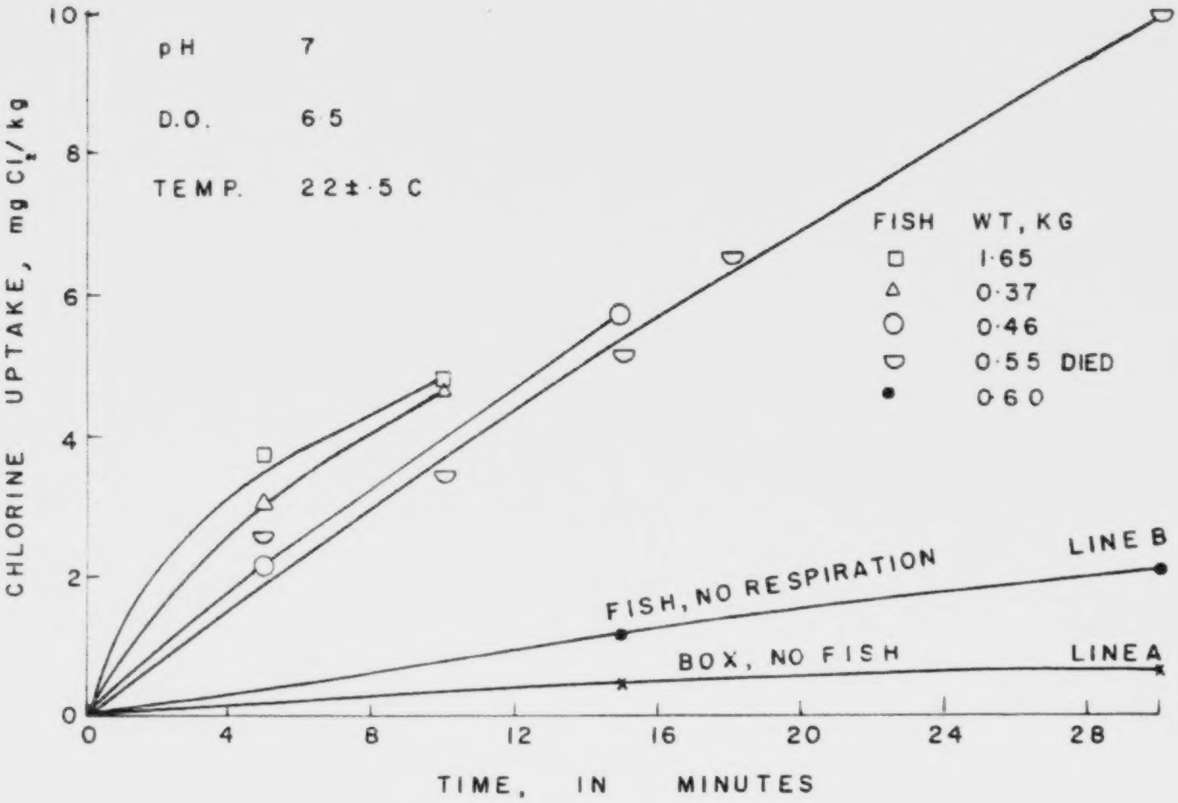

Figure 3 Chlorine uptake by five catfish. Four respiring fish had uptake rate averaging 0.4 mg Cl_2/min/kg. An anesthetized fish (no opercular movements) had an uptake rate of 0.05 Cl/min/kg. Loss of chlorine from the experimental system with no fish present was negligible.

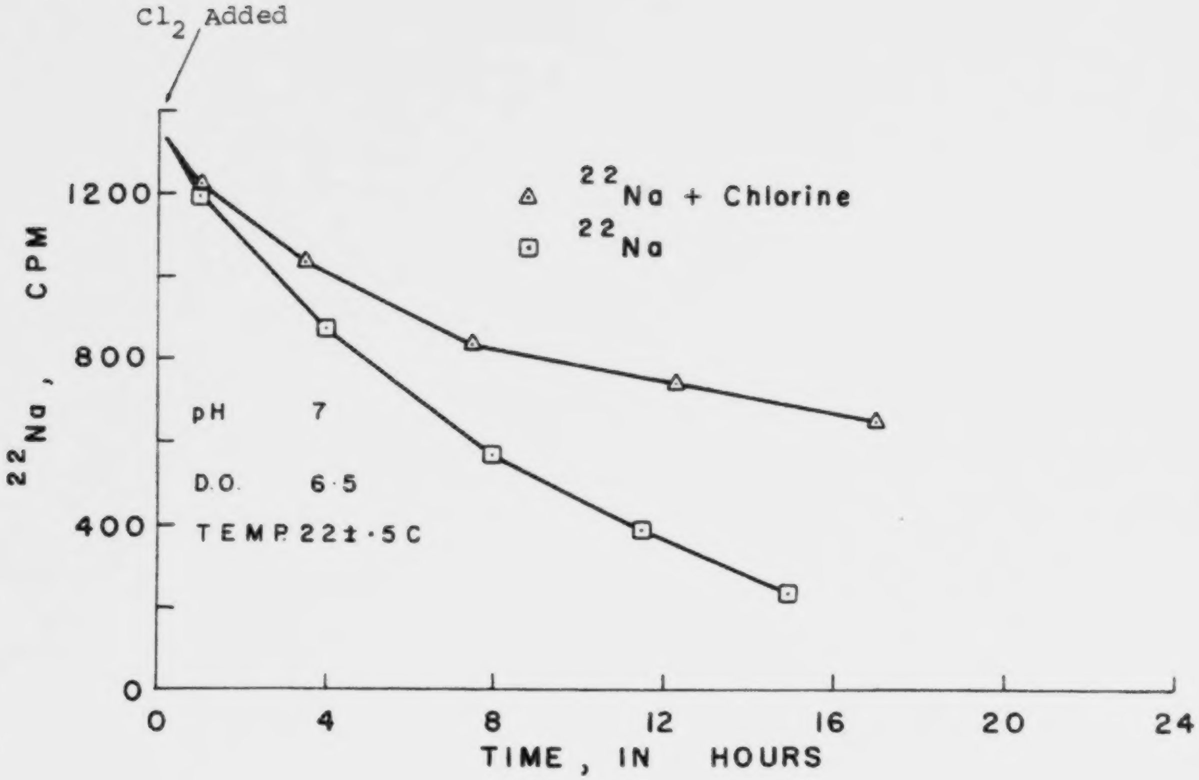

Figure 4 Effect of short term chlorine exposure on the uptake of ^{22}Na by the gills of *Ictalurus punctatus* (Fish #34, wt. 1.119 kg, and 0.1 mg/l of chlorine added at start of experiment).

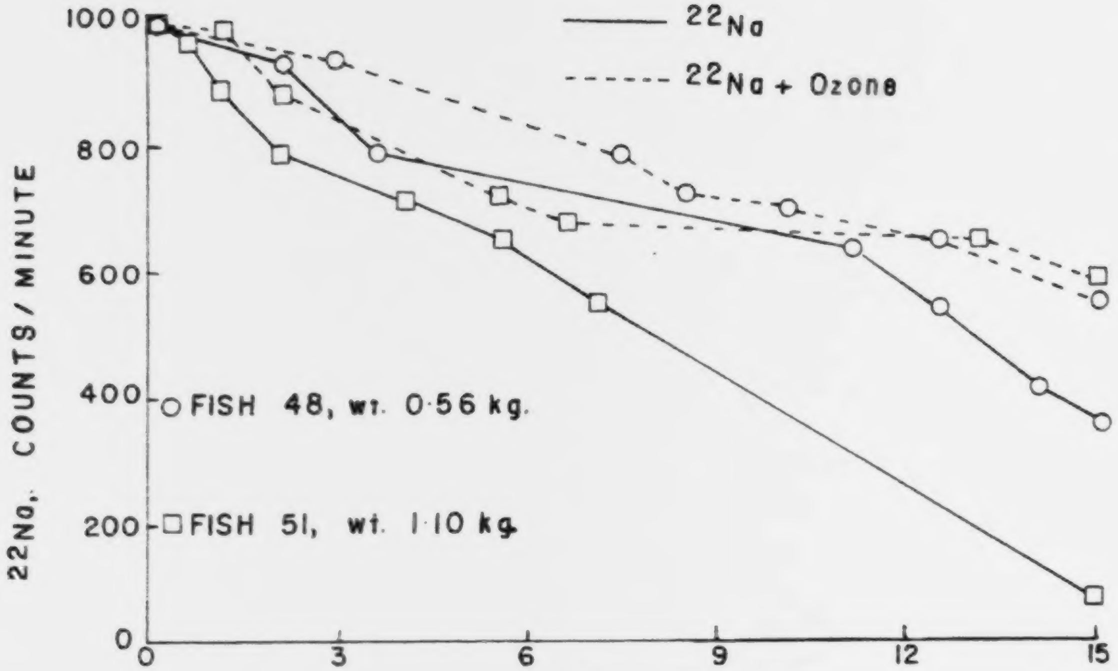

Figure 5 Effect of ozone exposure on the uptake of ^{22}Na by the gills of *Ictalurus punctatus*.

Heart Rate and Blood Pressure

The most dramatic physiological response to chlorine exposure was shown by heart rate and blood pressure. Blood pressure was continuously recorded prior to and following the continuous addition of chlorine to the flow through system. Figure 6 demonstrates the change in dorsal aortic pressure and heart rate after a 7.5 hour exposure to 0.22 mg/l chlorine. The introduction of chlorine caused an immediate drop in mean blood pressure, from 27 mm Hg to 17 mm Hg, and an initial decrease in heart rate of 50% from 55-60 beats/minute. This initial response is probably due to vagal inhibition directly affecting the heart. After 7.5 hours of chlorine exposure blood pressure dropped to 16 mm Hg, pulse pressure dropped to 2 mm Hg from 3 mm Hg and heart rate decreased to 40-45 beats/minute.

The exposure of fish to chlorine at levels approaching the 96 hr LC_{50} (0.07 mg/l chlorine) were immediately detected as shown by the pronounced drop in blood pressure (Fig. 7). Continued exposure for 5 hours resulted in an increase in heart rate from 22 beats/minute to 45 beats/minute. At an exposure of 0.03 mg/l chlorine there was no initial reaction shown by blood pressure or heart rate (Fig. 8). After 2 hours the fish appeared normal with no apparent disfunction.

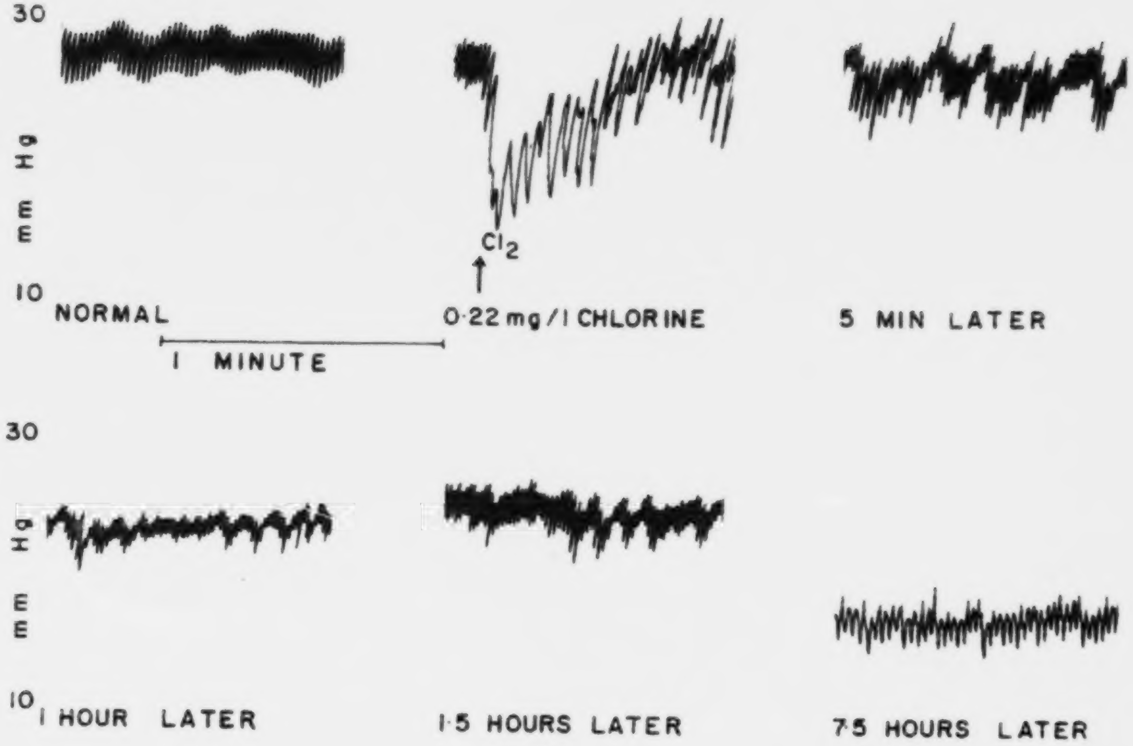

Figure 6 Influence of long term chlorine exposure on the blood pressure and heart rate of *Ictalurus punctatus* (Fish #38, wt. 0.832 kg).

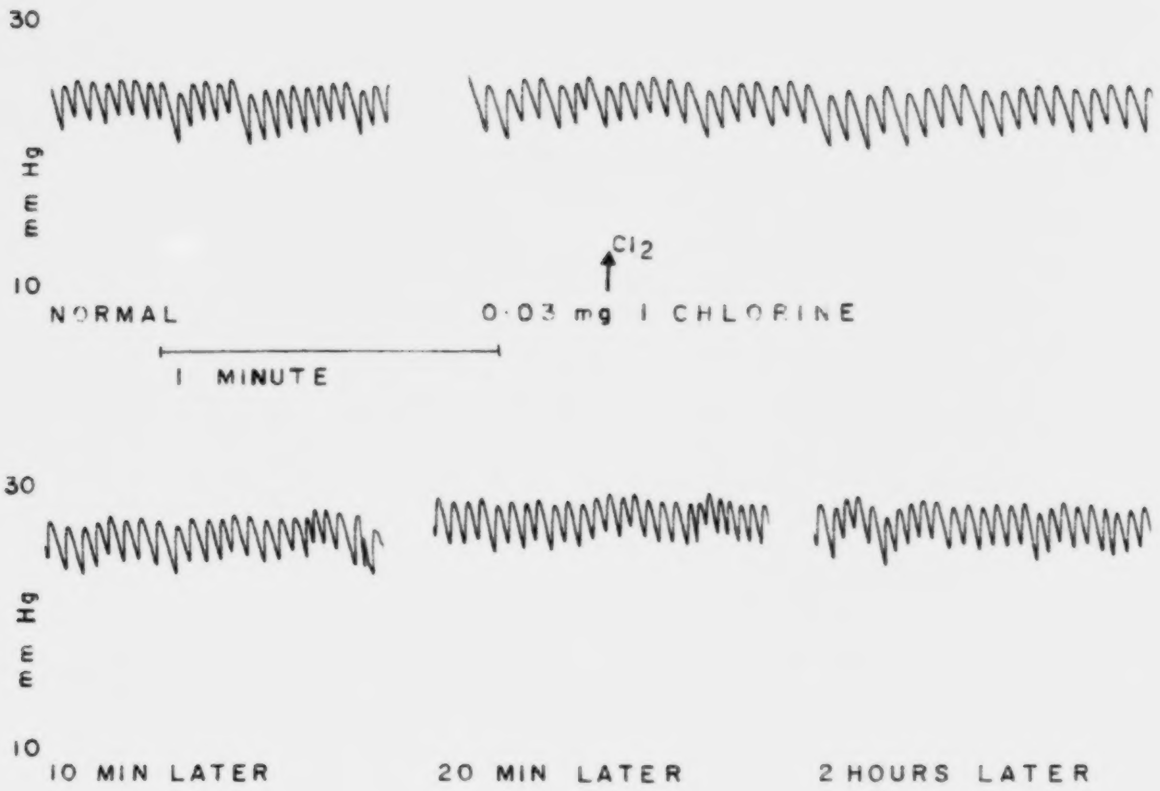

Figure 7 The influence of chlorine at a dose approximating the 96 hr. LC_{50} on heart rate and blood pressure of *Ictalurus punctatus* (Fish #54, wt. 1.544 kg).

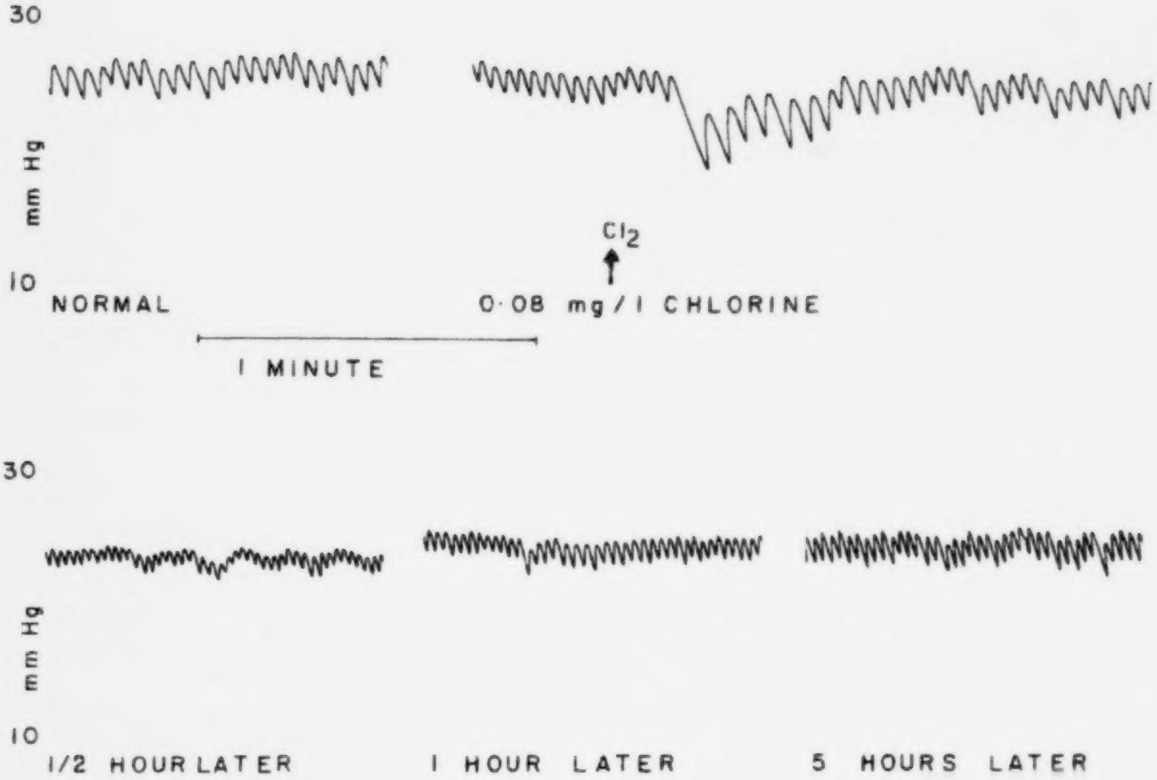

Figure 8 The influence of chlorine at a dose less than half of the 96 hr. LC_{50} on heart rate and blood pressure in *Ictalurus punctatus* (Fish #54, wt. 1.544 kg).

DISCUSSION

The effects of chlorine and ozone exposure on the physiological functions of *Ictalurus punctatus* have been examined. Those parameters evaluated included: kidney functions (ion excretion, urine flow, and glomerular filtration rate), gill sodium uptake, gill chlorine uptake, blood pressure and heart rate.

No alterations in renal functions were recorded. However, it is highly unlikely that long term exposure to chlorine would not have some negative effects. Because the kidneys are intimately involved as a homeostatic mechanism any alteration in surface permeability (body or gills) or ion exchange or carrier system would result in renal compensation. Any pollutant that is selectively concentrated or absorbed by the kidney may also cause tubular damage and result in disfunction. No evidence of renal tubular damage was revealed in the chlorine and ozone experiments conducted to date.

That the gills show a definite involvement in chlorine toxicity is also implied by the work of Block (1977). Chlorine is actively removed from the circulating water by the gills but is only minimally removed by the body surface (Fig. 3). The chlorine taken up by the gills has effect on the gill sodium transport mechanism (Fig. 4). A similar reduction in sodium transport was noted with ozone exposure. Thus it is concluded that any pollutant that affects ion secretory or absorptive cells should significantly affect body ion homeostasis since gill epithelium is an important extra-renal mechanism for electrolyte regulation (Conte, 1969). It should also be noted that these particular sodium transport experiments were conducted under ionic stress to enhance uptake and that long term chronic experiments under more natural conditions are required to verify the results.

The most significant physiological effects of chlorine exposure were changes in dorsal aortic blood pressure. Upon the introduction of chlorine the initial drop in blood pressure is dramatic (Figs. 6 and 7). Although vagal inhibition can occur from many stimuli, sham introductions did not produce the response. Although there is individual variation all fish displayed circulatory stress after 5-8 hours exposure to chlorine.

At concentrations approaching the 96 hr LC_{50} there was always an initial vagal inhibition. Normal blood pressures were maintained with only changes in heart rate being noted. At exposures approximately 1/2 the 96 hr LC_{50} (0.03 mg/l) no changes in physiological response were observed. There was no initial vagal inhibition and no change in blood pressure or heart rate during exposure for 24-48 hours. Thus it can be concluded that an exposure to a chlorine concentration of 0.007 mg/l (1/10 of 96 hr LC_{50} suggested by Horton, 1967) fish should show no physiological disfunction.

Although ozone was shown to impair gill sodium uptake similar to chlorine a very different response was observed on the circulation. At ozone concentrations from 1-2 mg/l the fish were aware of its presence and exhibited a reaction. However, it was noted during blood pressure experiments that the pronounced vagal inhibitions (initial drop in blood pressure) normally associated with chlorine exposure were not present with the introduction of ozone at lethal concentrations (0.1 - 0.5 mg/l). This leads to the conclusion that fish may be unaware of ozone at low lethal concentrations and any avoidance reaction may not take place.

For the purposes of hazard evaluation a wide range of physiological parameters were monitored. In this particular case renal function was not sensitive to short term chlorine or ozone exposure. Under ionic stress changes in gill sodium transport were apparent after several hours exposure. The most sensitive physiological response was the change in blood pressure following chlorine exposure. However, it must be noted that no pressure changes occurred following exposure to lethal ozone concentrations. Thus it is concluded that no single physiological parameter should be relied upon for a hazard evaluation.

ACKNOWLEDGEMENTS

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ABSTRACT

A continuous-flow apparatus was used to determine both acute and chronic effects of Trichlorobenzene (TCB) on discrete life stages of the American flagfish (Jordanella floridae). The 96 hour LC_{50} of TCB for one week old fry was 2.1 ppm.

Breeding communities of flagfish were exposed to a range of TCB concentrations (0.15-1.40 ppm) for 21 days. No adult mortality was recorded during the exposure period. However, there was a significant decrease ($p<.01$) in egg production above 0.28 ppm TCB and a termination of spawning above .80 ppm TCB. Excess control eggs were incubated at test levels where spawning had terminated. Hatching success was unaffected at all initial test levels. However, exposure of eggs to TCB concentrations of 2.32 ppm, and 2.72 ppm resulted in significant declines ($p<.01$) in hatching success.

Fry mortalities were significantly increased ($p<.01$) with increasing concentrations of 0.73, 1.71 and 2.32 ppm TCB. No fry survived the initial 24 hour period at 2.72 ppm TCB.

Results of the present study indicate TCB to be less toxic than PCB's by a factor of approximately 1,000.

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INTRODUCTION

Recent restrictions on the use of Polychlorinated biphenyls (PCB's) as a dielectric fluid, has resulted in a proliferation of proposed replacement compounds. Trichlorobenzene (TCB) is one of the suggested PCB-substitute compounds. Indeed, TCB is currently being used in Canada to "top up" existing PCB-filled transformers and as a result was the first of several replacement compounds being evaluated in our laboratory.

The present study was conducted to determine the toxicity of TCB to the American flagfish and to assess the chemical's suitability as an environmentally acceptable dielectric fluid.

MATERIALS AND METHODS

A flow-through system comprised of an equal volume diluter (DeFoe, 1975), a modified pneumatic injector (Smith, et. al., 1977) and 28, 30 ℓ test tanks was used for both acute and chronic testing.

The diluter delivered four liters of tempered (25°C) de-chlorinated water per cell. The injector delivered 1 ml of acetone carrier per cell. With the exception of acetone control, each 250 ℓ of carrier (196 ppm) contained a prescribed concentration of TCB. Water from the siphon initiator cell served as a water control. Cycle time was five minutes, providing a 90% replacement in approximately six hours (Sprague, 1969).

The test tanks consisted of a duplicate set of seven spawning tanks and a corresponding set of progeny tanks. Test tanks were individually equipped with immersion heaters to maintain water temperature at 26°C. A magnetically driven stirrer was placed in each test tank to assist in the mixing of the toxicant and the water.

Photoperiod was 16 hours of "daylight" with an additional 15 minute dawn-dusk simulation.

Test tank concentrations were measured on daily grab samples (250 ml). Hexane extracts of the water samples were analyzed with a Hewlett-Packard 5730-A gas chromatograph equipped with a ^{63}Ni electron-capture detector. Column packing consisted of 8% Bentone-34 and 10% DC-200 on 80-100 mesh GCQ. Column temperature was 178°C and the flow of carrier gas (5% argon in methane) was maintained at 50 ml/min.

Acute Testing

A standard 96-hour flow-through bioassay was used to establish an LC_{50} value for TCB. Nominal test concentrations of TCB were 2.4, 3.2, 4.2, 5.6 and 7.5 ppm. Ten, one-week old flagfish were placed in fry retainers (Murphy, 1978) located in each of the duplicate adult test tanks. Fry survival was checked at regular intervals and dead fry were recorded and removed. Fry were fed newly hatched brine shrimp (*Artemia salina*) once daily. An LC_{50} was calculated by probit analysis. A second test was conducted to confirm the initial LC_{50} value.

Chronic Testing

Juevenile male and female flagfish were randomly distributed among the duplicate spawing test tanks. All fish were maintained in non-dosed "control" water for a one month growth and maturity period.

Diet consisted of frozen adult brine shrimp (*Artemia salina*) fed twice daily. Excess food and debris were siphoned from the tanks daily.

Following maturation, the fish were selectively called (i.e., similar size fish of both sexes were retained) to a ratio of five females to two males per tank and spawning substrates were introduced. The substrates were removed daily and examined for eggs. Clean substrates were immediately substituted for those removed. Eggs were separated from the spawning substrate, examined under a microscope and unfertile or abnormal eggs were discarded. A maximum of 50 fertile eggs from each spawn were placed in individual egg cups and treated with malachite green (4 mg/l) for five minutes. The egg cups were then attached to a rocker arm assembly (Mount, 1968) in their respective progeny tanks.

Once synchronous spawning in all test tanks was achieved, toxicant addition was initiated. Nominal TCB concentrations were: 0.32, 0.56, 1.0, and 3.2 ppm, delivered in 250 µl/l of acetone. Stable test tank concentrations of TCB were reached within the initial 24-hour dosing period and measured daily for the duration of the 21-day experimental period.

Excess control eggs were transferred and incubated at TCB test concentrations where spawning had terminated.

In a second experiment, control eggs were exposed to a new nominal TCB concentration gradient of 1.8, 3.2, 5.6 and 7.5 ppm. In addition to water and acetone controls, eggs incubated at nominal TCB concentrations of 1.8 and 3.2 also served as controls against previous incubation data.

Fifty fry were exposed to the same nominal TCB concentration gradient to determine fry survival over a 10-day period. All fry were acquired from control eggs incubated at the nominal TCB concentrations. Fry were transferred from the egg cups to fry retainers within 48 hours following hatching. Newly hatched brine shrimp fed once daily, served as a diet.

Differences in egg production, viability and hatching success at different TCB concentrations, were tested for significance by a oneway analysis of variance. If a significant F-ratio was found, a Duncan multiple range test (Duncan, 1955) was employed to determine which test concentration yielded significantly different results at the .01 and .05 test levels. Fry survival was subjected to Chi-square analysis.

RESULT AND DISCUSSION

Measured TCB concentrations were approximately 50% of the nominal concentrations. The per cent recovery decreased with increasing nominal concentrations (Table 1). Loss to the atmosphere, in addition to "plating out" of the compound on the walls of the receiving vessels, account for this substantial loss. Considering the volatility and low water solubility of TCB, measured test tank concentrations were very consistent (Table 1).

Results of the acute bioassays are presented in Table 2. Both tests one and two yielded similar 96 loss LC_{50} values, 2.1 and 2.2 mg/ℓ respectively. The 96 Loss LC_{50} for Aroclor 1242 is reported to be 15 μg/ℓ for newly hatched fathead minnows, *Pimephales promelas* (Nebeker, et al., 1974). Although the exposure time was extended to 21 days, Aroctor 1254 appears to be more toxic with a recorded LC_{50} value of 0.93 μg/ℓ for the fry of sheepshead minnows, *Cyprinodon variegatus* (Schimmel, et al., 1974). One can also see an increase in sensitivity with time, ranging from 2.32 ppm TCB at 48 hours to 1.92 ppm at 144 hours (Table 2). Juevenile pinfish, responded in the same manner when exposed to Aroclor 1016 (Hansen, et al., 1974).

No adult mortality occured at any test level during the three week exposure to TCB. There was however, a decrease in general activity and feeding intensity at 1.5 ppm TCB. Similarly, adult sheepshead minnows became very lethargic at sublethal concentrations of Aroclor 1254 (Schimmel, et al., 1974).

There was a significant decrease ($p<.01$) in egg production at 0.29 and 0.48 ppm TCB and a termination of spawning at 0.81 and 1.5 ppm TCB (Table 3). Fathead minnows failed to spawn in concentrations exceeding 1.8 μg/ℓ of A-1254 and 5.4 μg/ℓ of A-1242 (Nebeker, et al., 1974). At TCB concentrations where spawning occurred, viability was high (>95%) (Table 3). However, with so few eggs produced at 0.93 and 1.71 ppm TCB, the viable egg data may not be representative of exposure to these concentrations.

Exposure at and above 1.71 ppm TCB resulted in a significant decline ($p<.01$) in hatching success. There was also a significant reduction ($p<.01$) in hatching success with increasing concentration between 1.71 and 2.72 ppm TCB (Table 3). Hatching success of sheepshead minnow eggs was significantly reduced at 10.0 μg/ℓ of A-1254 (Nebeker, et al., 1974). Nebeker, et al. (1974) found the hatching success of fathead minnow eggs increased with increasing concentration up to 5.4 μg/ℓ of A-1242. This was attributed to the inhibition by PCB of bacterial and fungal growths. The movement of water provided by the rocker arm assembly, as well as as the treatment in malachite green, probably eliminated this hatching phenomenon in the present study.

No fry survived the initial 24 hour exposure period at 2.72 ppm TCB. Fry retained their embryonic curvature following hatching at 2.32 and 2.72 ppm TCB. There was very little locomotor activity and as a result, greatly reduced feeding activity at 1.71 ppm TCB. Virtually no feeding activity occurred at 2.32 ppm TCB. This is reflected in the fry mortality rate (Table 3, Fig. 1) with low initial mortality followed by an increased mortality rate after eight days exposure at 1.71 ppm TCB and six days exposure at 2.32 ppm TCB. Fry mortality was not affected at 0.93 ppm TCB, however, fry mortality was

TABLE 1. Test tank concentrations of trichlorobenzenze (TCB) during the exposure of discrete life stages of the American flagfish

Acute

Nominal Concentration	Test #1. Measured test tank concentration	Test #2. Measured test tank concentration
2.4	1.67 ± 0.19	1.45 ± 0.13
3.2	1.95 ± 0.22	1.65 ± 0.21
4.2		2.21 ± 0.20
5.6	2.60 ± 0.26	2.40 ± 0.31
7.5	3.61 ± 0.10	3.08 ± 0.16

Chronic

	Adult tanks		Progeny tanks	
Nominal Concentration	Measured test tank concentration	% Recovery	Measured test tank concentration	% Recovery
0.32	0.15 ± 0.01	48.7 ± 4.66	0.22 ± 0.01	68.0 ± 3.74
0.56	0.29 ± 0.03	51.9 ± 5.31	0.34 ± 0.02	60.2 ± 3.11
1.0	0.48 ± 0.08	47.5 ± 7.49	0.55 ± 0.05	54.6 ± 4.83
1.8	0.81 ± 0.11	44.9 ± 6.33	0.93 ± 0.20	51.8 ± 11.17
3.2	1.50 ± 0.20	46.0 ± 6.12	1.71 ± 0.10	53.4 ± 2.88
5.6	2.60 ± 0.70	45.6 ± 12.6	2.32 ± 0.31	41.5 ± 5.50
7.5			2.72 ± 0.32	36.3 ± 4.20

Note: All test concentrations are reported in mglℓ. TCB was delivered in acetone carrier (196 ppm)

TABLE 2. Acute toxicity of trichlorobenzene (TCB) LC_{50} values for one week old flagfish (*Jordanella floridae*) fry

Exposure time	Test #1 LC_{50} value (ppm)	Test #2 LC_{50} value (ppm)
48 hr.	2.40 (2.214-2.608)	2.32 (2.286-2.364)
72 hr.		2.26 (2.163-2.372)
96 hr.	2.11 (1.899-2.335)	2.21 (2.105-2.319)
120 hr.	1.99 (1.796-2.216)	1.94 (1.836-2.045)
144 hr.	1.92 (1.761-2.098)	

Note: Data subjected to probit analysis. TCB delivered in acetone carrier (196 ppm).

TABLE 3. Reproductive response of flagfish (*Jordanella floridae*) exposed to trichlorobenzene (TCB) for 21 days.

			Nominal test concentration (ppm)						
Parameter	Cont.^{1}	Acetone^{2} cont. (196 ppm)	0.32^{3}	0.56^{4}	1.0^{5}	1.8^{6}	3.2^{7}	5.6^{8}	7.5^{9}
Spawnings	38	39	41	27	15	3	3		
Total eggs	2088	2725	2945	561	252	6	11		
$\bar{x}$ Daily spawn	49.7 ±43.7	64.9 ±59.9	70.1 ±54.1	13.4 ±28.0	6.0 ±13.3	0.14 ±0.65	0.26 ±1.40		
$\bar{x}$ % viable	99.5 ±1.2	98.6 ±2.4	99.0 ±1.7	99.1 ±3.6	99.1 ±3.4	100 ±0.0	963 ±6.4		
$\bar{x}$ % hatch	96.0 ±2.8	98.0 ±2.3	99.5 ±1.0	97.0 ±3.8	96.3 ±3.7	96.7* ±3.0	93.3 ±9.7	91.0* ±3.8	60.5* ±2.5

ANOVA - $\bar{x}$ Daily spawn F = 30.4 range test 3 2 1 4 5 7 6

ANOVA - $\bar{x}$ % viable F = 1.1 range test 6 1 5 4 3 2 7

ANOVA - $\bar{x}$ % hatch F = 1583.3 range test 3 2 4 6 5 1 7 8 9

Note: Numbered means (arranged in ascending order) not underscored by the same line are significantly different at the 1% level. Data obtained from duplicate banks has been grouped.

* control eggs

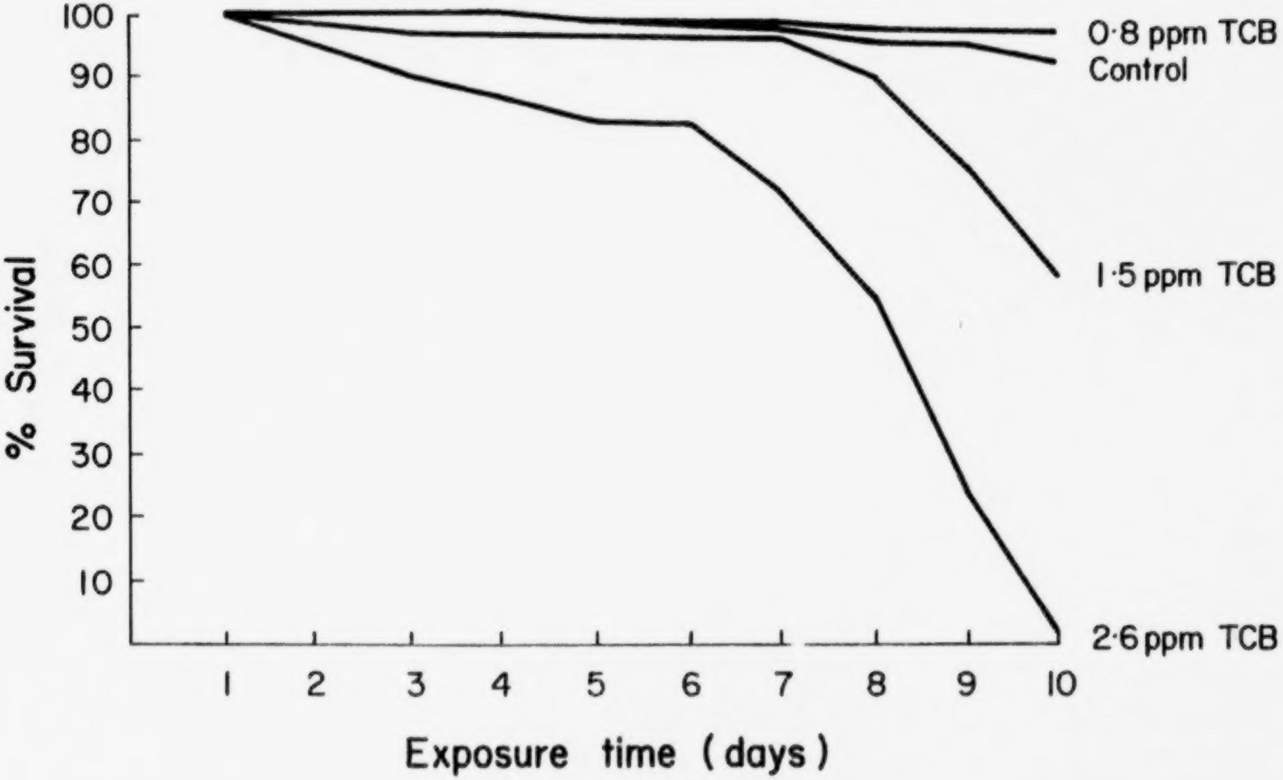
TABLE 4. Flagfish Fry Survival Exposed to trichlorobenzene for 10 days

	Measured TCB Concentration (ppm)			
Day	Cont.	0.81	1.50	2.60
1	100	100	100	100
2	100	100	99	94
3	100	100	97	90
4	100	100	97	87
5	99	99	97	83
6	98	99	97	83
7	98	99	96	72
8	96	98	90	56
9	96	98	77	24
10	93	98	59	1

Chi-square analysis of 10 day mortality lebels $\underline{93\ \ 98}\ \ \underline{59}\ \ \underline{1}$

Note: Values not underscored by the same line are significantly different at the 1% level.

Fig. 1. Survival of flagfish fry exposed to trichlorobenzene for 10 days.



significantly increased ($p<.01$) at 1.71 and 2.32 ppm TCB. In addition, fry mortality was significantly increased ($p<.01$) with increasing concentration between 0.93 and 2.32 ppm TCB. Sheepshead minnow fry exposed to A-1254, showed a significant increase in mortality at 0.32 µg/ℓ (Schimmel, et al., 1974), where as fathead minnows exhibited good survival in concentrations as high as 1.8 µg/ℓ (Nebeker, et al., 1974). Aroclor 1248 was slightly less toxic to flagfish fry where no significant increase in mortality was observed up to concentrations of 2.2 µg/ℓ.

Results of the present study indicate TCB to be less toxic than PCB's by a factor of approximately 1,000.

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ABSTRACT

The recent detection of pentachlorophenol (PCP) residue in water and watersheds of the Great Lakes indicates that significant quantities of this toxicant are being discharged into the environment, particularly from sewage treatment plants.

The toxicity of chlorophenols to the microorganisms of activated sewage sludge were investigated. Phenol, O-chlorophenol, 2,6-dichlorophenol, 2,4,6-trichlorophenol (TCP) and pentachlorophenol (PCP) were added to bacterial cultures at concentrations between 2-100 ppm.

Addition of phenol at concentration of 20-100 ppm stimulated bacterial growth, while TCP and PCP inhibited at concentrations as low as 2 ppm and 50 ppm of these substances caused 100% mortality of the cells.

The extent of inhibition is directly related to the degree of chlorination in the aromatic ring.

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INTRODUCTION

Chlorinated phenols are commonly used as broad spectrum biocides (Geisser and Garver, 1977; Hegna, 1977) for example, pentachlorophenol (PCP) has been used as a wood preservative in forest products in large tonnages for about six decades (Venkobachar *et al*. 1976). Recent works (Burtschell *et al*. 1959; Koziorowski and Kucharski, 1972) have indicated that chlorinated phenols could also be produced through the chlorination of sewage and other waste disinfection processes.

These high and wide-spread PCP 'usages' suggest that this compound could be widely distributed in the environment, indeed, trace amounts of PCP have been detected in municipal water supplies, wells and human beings (Rivers, 1972). Recently, detection of PCP residues in the waters of the Great Lakes indicates that significant quantities of this toxicant are being discharged into the environment from industrial processes, agricultural uses, and sewage treatment plants (Fox, 1978).

Since activated sludge processes are commonly used in municipal sewage treatment for the reduction of organic matter, it is important to ensure that the concentration of PCP in raw sewage effluent will not interfere with the normal operation of such processes which, in turn, will affect the discharge of organic substances to the lakes. Consequently a laboratory study was undertaken to determine the toxic effects of some chlorinated phenols to microorganisms from activated sludge. In addition, these tests were routinely used in our biodegradation experiments to ensure that the concentrations of the test chemical were not inhibitory to the microorganisms.

MATERIALS AND METHODS

Microorganisms: Activated sludge from the Burlington municipal sewage treatment plants was used as bacterial inoculum. One ml of fresh activated sludge was added to 100 ml of modified nutrient broth in a 250 ml. Erlenmeyer flask. The mixture was incubated on a gyrotory shaker (120 strokes/min) at room temperature (22°C) for 18-20 hrs. Then this culture was used for bacterial seedings in the subsequent toxicity tests. Routine examination of the activated sludge, as well as the culture mixture under a phase microscope was also employed to make sure that no significant change of bacterial types took place during the experiment.

Medium: The "Difco Nutrient Broth", fortified with 0.2 g/L each of glucose and Na-acetate was used as the test medium. Our experience indicated that this medium would support more heterotrophic bacterial growth than the nutrient broth alone. The medium was sterilized at 121°C for 15 min.

Chemicals: Phenol, chlorophenol (CP), 2,6-dichlorophenol (DCP), 2,4,6-trichlorophenol (TCP) and pentachlorophenol (PCP) were tested. Stock solutions of chemicals (1% and 4%) were prepared by dissolving the chemicals in 0.5 N NaOH and adjusting the pH to 9.0. The solutions were sterilized by membrane filtration.

Growth Study: An aliquot of 0.1 ml of appropriate dilution blank containing 2×10^4 cells/ml from the stock bacterial suspension was added to 125 ml sidearm flasks containing 20 ml of the modified nutrient broth with and without the addition of various amounts of phenol and chlorinated phenols. Ranges of resultant concentrations for phenol were 20-100 ppm, and for the chlorophenols 2-100 ppm were used. The flasks were incubated on gyrotory shaker (120 strokes/min) at 22°C for 90 hours. At selected time intervals, the turbidity in each flask was measured using a Klett Summerson colorimeter equipped with a red filter.

RESULTS AND DISCUSSION

Figure 1 shows the growth curve of the sewage bacteria (activated sludge) as effected by the various chlorinated phenols at the concentration of 2 ppm. From the figure it can be seen that the bacterial cells were more susceptible to the inhibitory effect of chlorinated phenols during the lag growth phase. However in order to make proper measurement of bacterial growth rate, the lag growth phase between 16-20 hrs were arbitrarily chosen. Assessment during this time seemed desirable as it would increase the sensitivity of the test and so eighteen hours incubation time was chosen for growth rate determinations. After 20 hrs the inhibitory effect was not so apparent. Moreover, phenols toxicity is not a constant but depends on various parameters, particularly the accumulation of toxic metabolic wastes and as organic acids.

Prolonged periods of incubation such as 48 and 96 hrs may result in adaptation processes with bacteria likely to increase in resistance to the toxic chemicals to which they are exposed. Although most chlorophenols at concentrations of less than 20 ppm did not significantly effect the growth rate of sewage bacteria even at these times, changes in bacterial types were observed in flasks containing higher concentrations of chemicals. At 50 ppm vegetative bacteria were severely inhibited by all chlorinated phenols and spore forming bacteria predominated. The relative toxicity to sewage microorganisms of the chlorophenols at 10 ppm indicated that they behaved qualitatively the same as it was observed at 2 ppm.

Figure 2 shows the percentage of inhibition effects due to the degree of chlorination in the phenol molecule. A relationship is observed between the degree of chlorination and the toxicity - the higher chlorinated phenols being more toxic to the sewage microorganisms. However, phenol itself at concentrations of 20-100 ppm was found to stimulate the growth rate of sewage bacteria and this stimulation mechanism cannot be explained here with the limited data.

Since PCP is the most toxic and persistent phenol among the chlorinated phenols (Adelman *et al*. 1976; Ingols and Stevenson, 1963), particular attention was paid to the inhibitory effect of PCP. The results of 2-50 ppm concentrations are shown in Figure 3. It can be seen that PCP added to the bacterial culture at the concentration of 50 ppm inhibited 100% of bacterial growth. This toxic effect was probably due to its ability to inhibit the essential metabolic process of oxidative phosphorylation (Weinbach, 1956; Lyr and Ziegler, 1959).

From this study and the observed levels of chlorophenols in sewage by Buhler *et al*. (1973), it can be concluded that the level of chlorinated phenols in raw sewage will not generally interfere with the sewage treatment process.

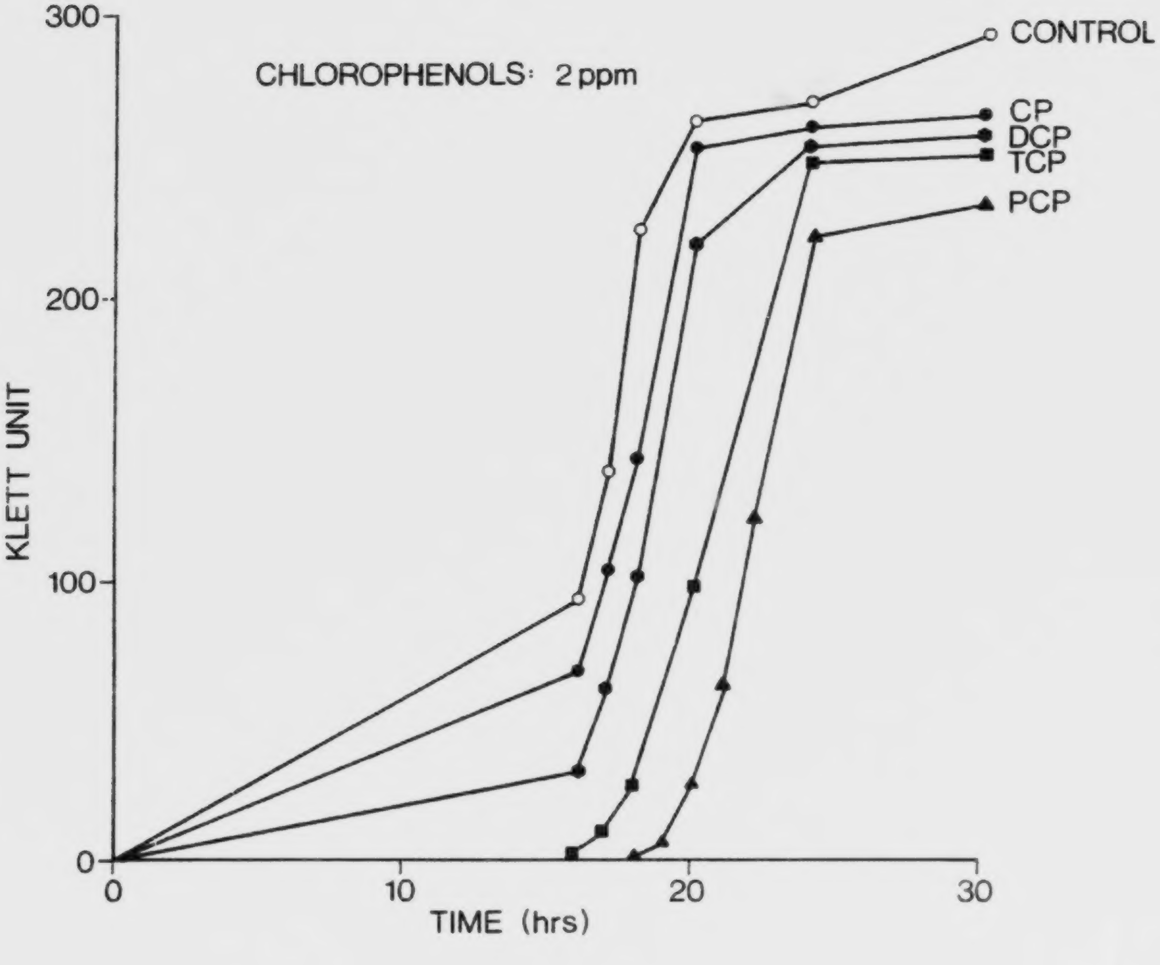

Figure 1: Growth of sewage bacteria effected by chlorophenols.

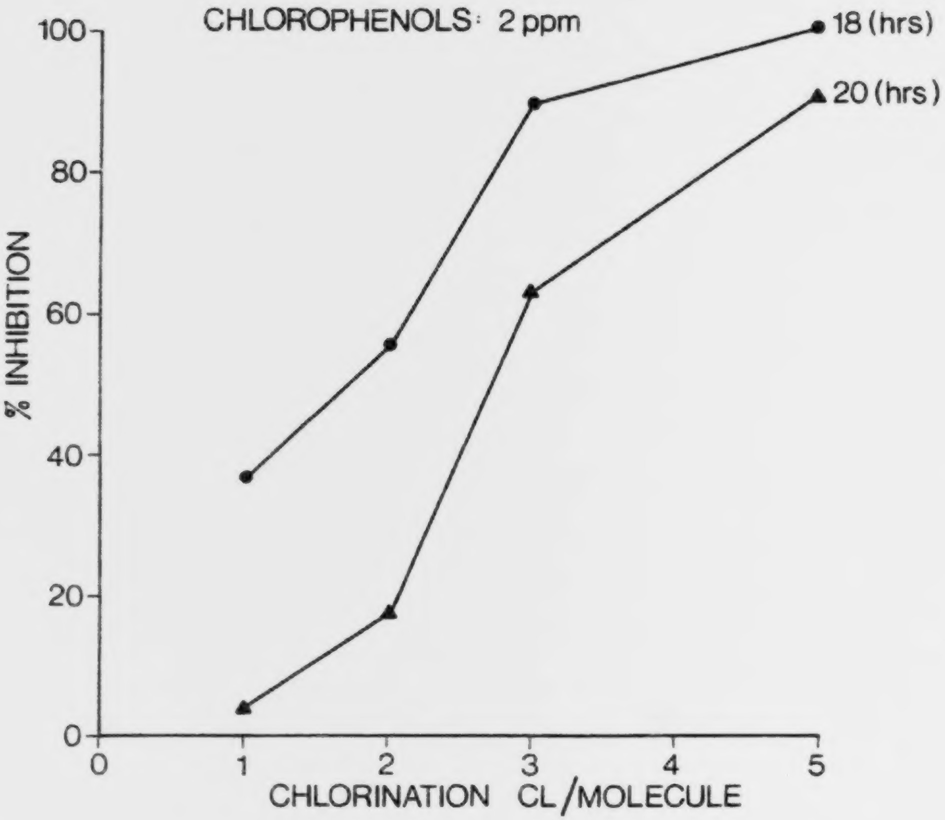

Figure 2: Percent inhibition as a function of extent of phenol chlorination. CP(1); DCP(2); TCP(3) and PCP (5 chlorinated a molecule)

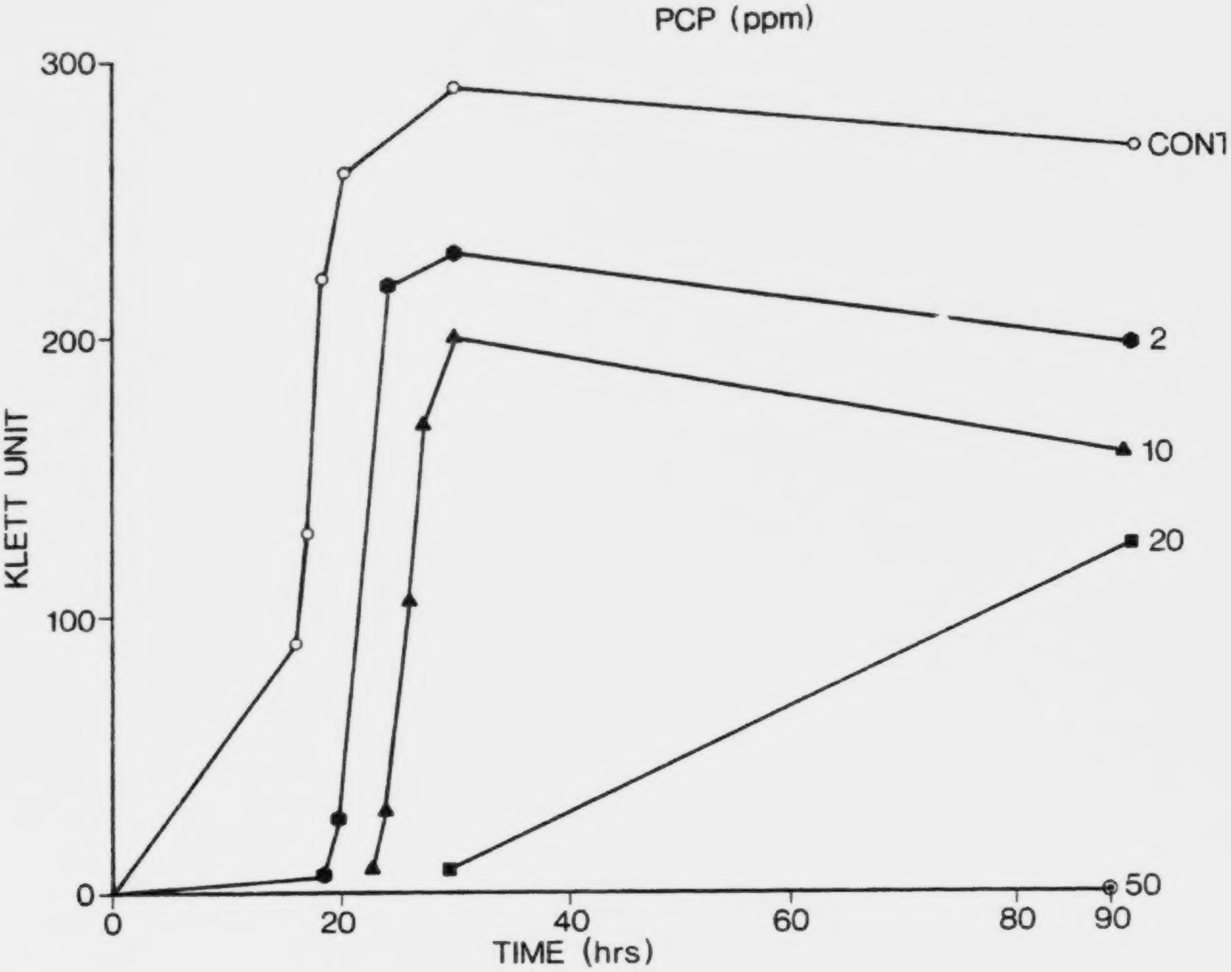

Figure 3; Effect of pentachlorophenol on bacterial growh rates.

●–● 2 ppm
▲–▲ 10 ppm
■–■ 20 ppm
○–○ 50 ppm

Concentrations of PCP are usually in the ppb range (Buhler *et al*. 1973) and it is not likely that other chlorinated phenols will raise this much. This conclusion assumes there are no synergistic effects. The toxicity of chlorophenols to bacteria could be determined by the other methods (Broecker and Zahn, 1976) of greater sensitivity, but the turbidity test is an easy and fast indicator for use in routine work. Results can be obtained within 24 hours and therefore it is useful for determining gross toxicity. It is also of value in ensuring that the results obtained in other microbial studies, particularly on degradation, do not reflect toxicity as opposed to other influences.

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ABSTRACT

Changes in level and pattern of locomotor activity caused by environmental toxicants have been repeatedly reported, but techniques to record and analyse activity of aquatic organisms are not yet well developed.

Inaudible high frequency sound is known to penetrate water very well. Ultrasonic transducers over a wide range of specifications have become available at moderate cost. Therefore, it appeared promising to develop an actograph based on the interruption of sonar beams.

The sonar frequency selected was 200 kHz. Tests demonstrated that rainbow trout from 20 to 60 cm length, when crossing a beam, caused the signal levels to drop on the receiving end of the beam by 90 to 99 per cent.

Two prototype test systems were designed. In the laboratory version, the incidence of beam interruptions (a measure of activity) was automatically translated into ASCII (American Standard Code for Information Interchange) and transferred to punched paper tape for plotting and processing via an IBM 370/168 terminal. For the field version, a simpler, less power-consuming battery-operated recorder with digital printout was developed.

These two test devices allow continuous and automatic day-and-night monitoring of an essential, highly integrative, biological response under both laboratory and field conditions.

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INTRODUCTION

Among sublethal responses to toxicants, changes in level and pattern of locomotor activity are of particular interest for 3 reasons:

1) Locomotor behaviour represents a high degree of integration, providing information on toxic effects at the whole-animal level (Scherer, 1977).

2) Largely overlooked until the 1930's, the ecological importance of locomotor activity patterns fitting the organism into temporal niches for optimal availability of biotic (e.g. food) or abiotic factors (light, temperature etc.) has since increasingly become apparent (Frisch et al., 1961; Solberger, 1965; Brown et al., 1970; Schwassmann, 1971).

3) In recent years, a number of authors have demonstrated significant effects of environmental toxicants on levels and patterns of locomotor activity (Waller and Cairns, 1972; Weiss and Weiss, 1974; Bengtsson, 1974; Ellgaard et al. 1977; Percy and Mullin, 1977).

Our paper will describe and discuss the rationale for and principle of a new approach to automatically record activity changes caused by toxicants under laboratory and field conditions. Details for construction, lists of parts and prices are available in a technical report (Scherer et al. 1977) and a forthcoming publication by Nowak et al.

WHY SONAR BEAMS?

A widely used technique for recording activity deploys light beams in the test area. Their interruption by moving animals, as registered through photocells connected to recorders, yields measures of activity.

Preferably, these light beams consist of wavelengths invisible to the test species (i.e., infrared or ultraviolet), so as not to influence their spatial distribution or circadian (light-controlled) activity rhythm.

This light beam method has also been applied to aquatic animals (e.g. Waller and Cairns, 1972; Bengtsson, 1974; Wallace et al., 1975). High absorption and scatter of light in water, however, limits the feasibility of this approach considerably. This holds true especially for infrared light even in pure (distilled) water, and more so in natural and contaminated waters for both infrared and ultraviolet.

In contrast, inaudible high-frequency sound is known to penetrate water very well. Technical developments in recent years have made ultrasonic transducers (with a wide range of specifications) available at moderate cost. Therefore, it appeared promising to develop an actograph for aquatic organisms based on the interruption of sonar beams.

TWO PROTOTYPES

Two satisfactory prototypes were developed in our laboratory. One is AC-line operated and equipped with an ASCII (American Standard Code for Information Interchange) tape punch system; the other is a battery-operated version with a simpler digital print-out developed for field use, such as on-site toxicant testing and monitoring (Figure 1).

In both designs, the sonar beams being interrupted by test animals are essentially identical. They are generated by pairs of transducers facing each other, with one transmitting at a frequency of 200 kHz, and the other receiving. This frequency was chosen for being safely (about 1 - 2 orders of magnitude) above the maximum perceived by most fish species (Popper and Fay, 1973).

Tests demonstrated that rainbow trout from 20 to 60 cm in length caused received signal levels (voltages at receiving transducer) to drop by 90 to 99% when crossing a beam. An electronic interface module, again essentially identical for the two prototypes, monitors received voltages and can be set to respond only to fluctuations exceeding those caused by internal electronic noise, or by floating debris and small air bubbles in the water.

A basic difference between the two prototypes relates to the recording method.

For laboratory use, we chose a tape punch system operating on ASCII to allow for automated data processing and plotting via an existing IBM 370/168 terminal (Figure 2).

For field application, the goal was to develop a battery-operated system with the lowest possible power consumption to allow the longest possible continuous on-site operation. This aspect prohibited an elaborate tape punch set-up. An initial solution was to use a modified spring-wound 24 hr temperature recorder which provided a blip on the circular chart for each beam interruption. However, this method resulted in very poor resolution of individual data points, especially at times of peak activity. An accurate and reliable recorder was developed by connecting to the timing and the voltage monitoring module an inexpensive, portable printing calculator. Information provided on the print-out consists of the number of interruptions per pre-set time interval and the time of day in hours and minutes at the moment of printout. These data can be used for manual processing and plotting (Figure 3), or be transferred onto punch cards for computer handling.

Cost of materials for a field unit consisting of 6 beams complete with voltage monitoring interface, timer and recorder was about $1,000.00; the tape punch equipment used in the laboratory version would add approximately $2,500.00.

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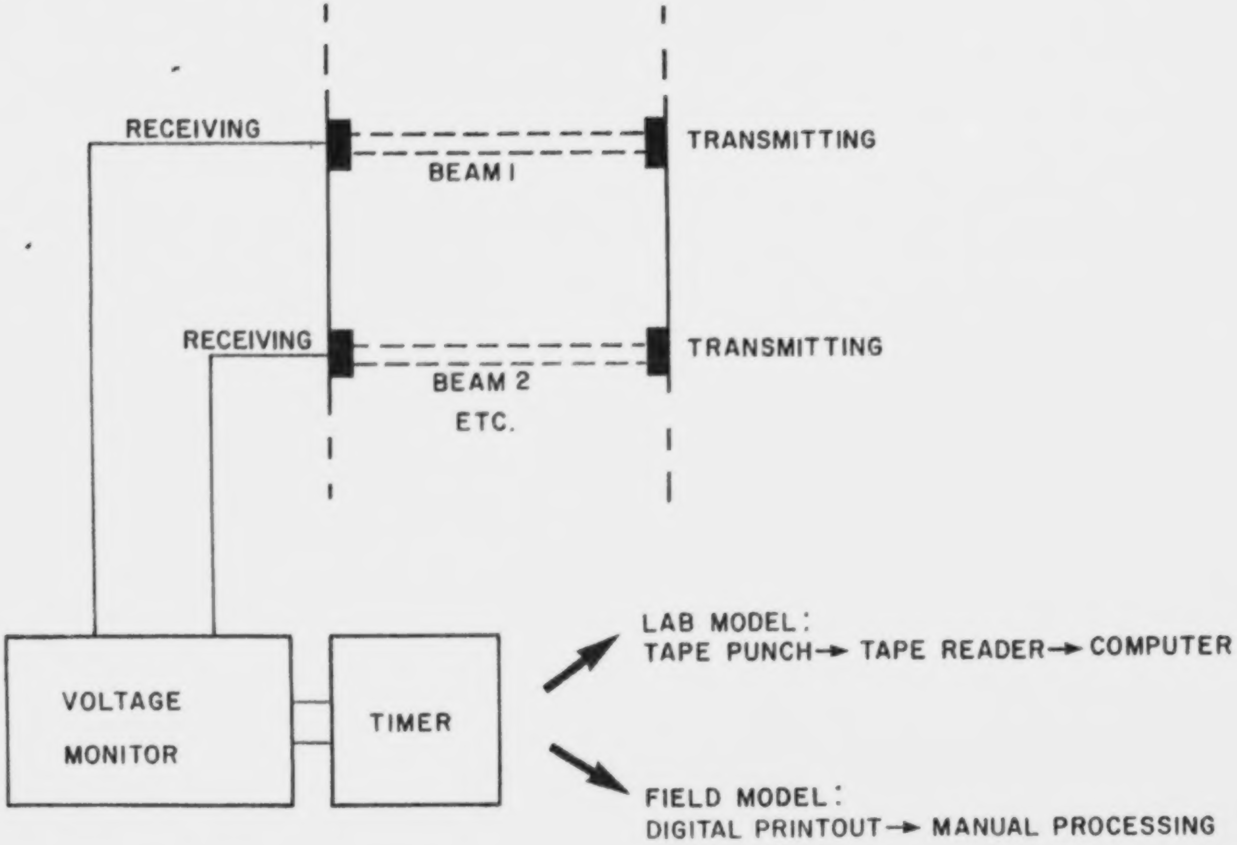
RECEIVING
TRANSMITTING
BEAM 1
RECEIVING
TRANSMITTING
BEAM 2
ETC.
VOLTAGE MONITOR
TIMER
LAB MODEL:
TAPE PUNCH → TAPE READER → COMPUTER
FIELD MODEL:
DIGITAL PRINTOUT → MANUAL PROCESSING

Figure 1. Schematic of laboratory and field version of sonar activity tester.

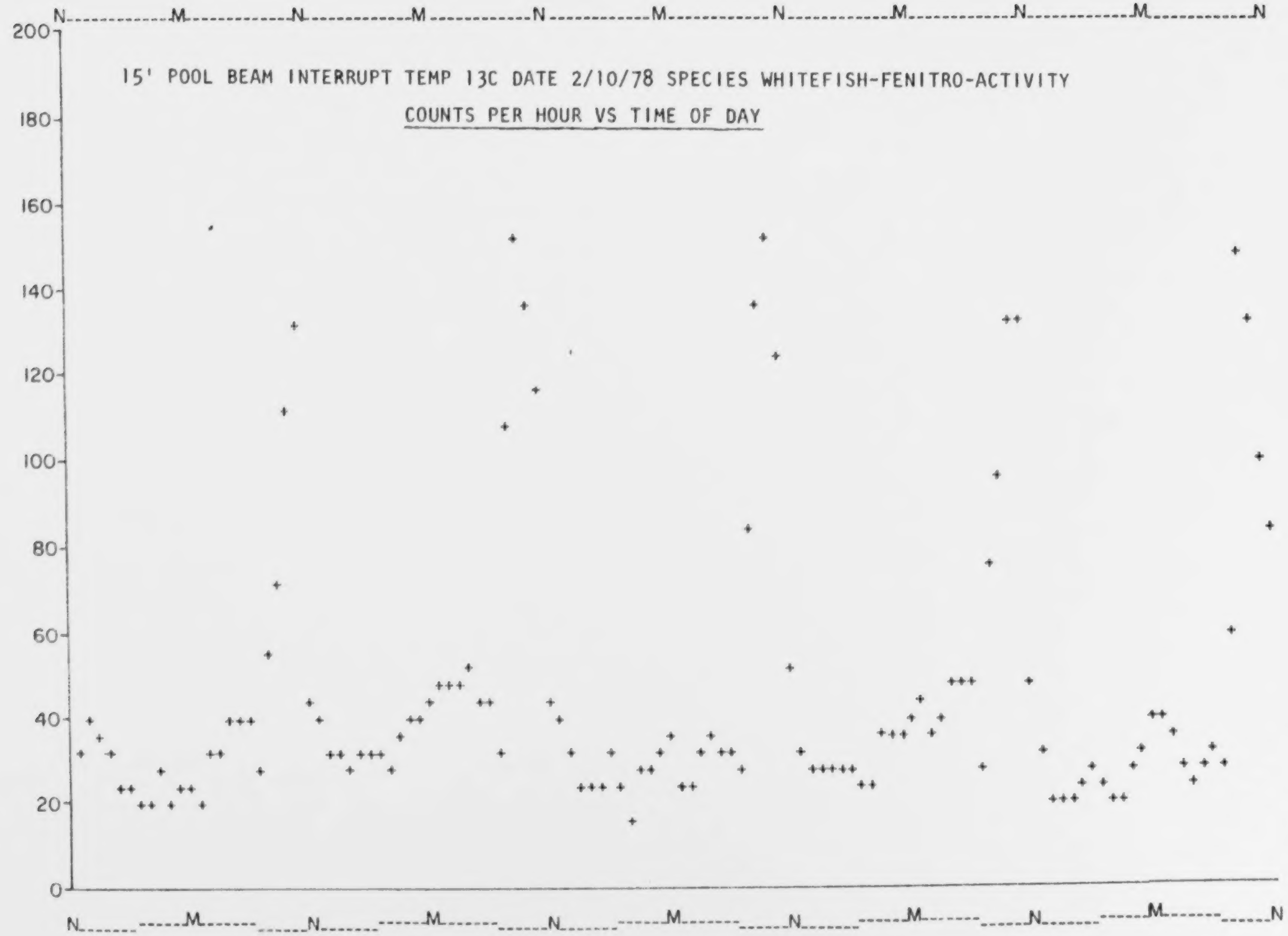

Figure 2. Example of laboratory model application. Computer printout of circadian activity pattern of whitefish (Coregonus clupeaformis)

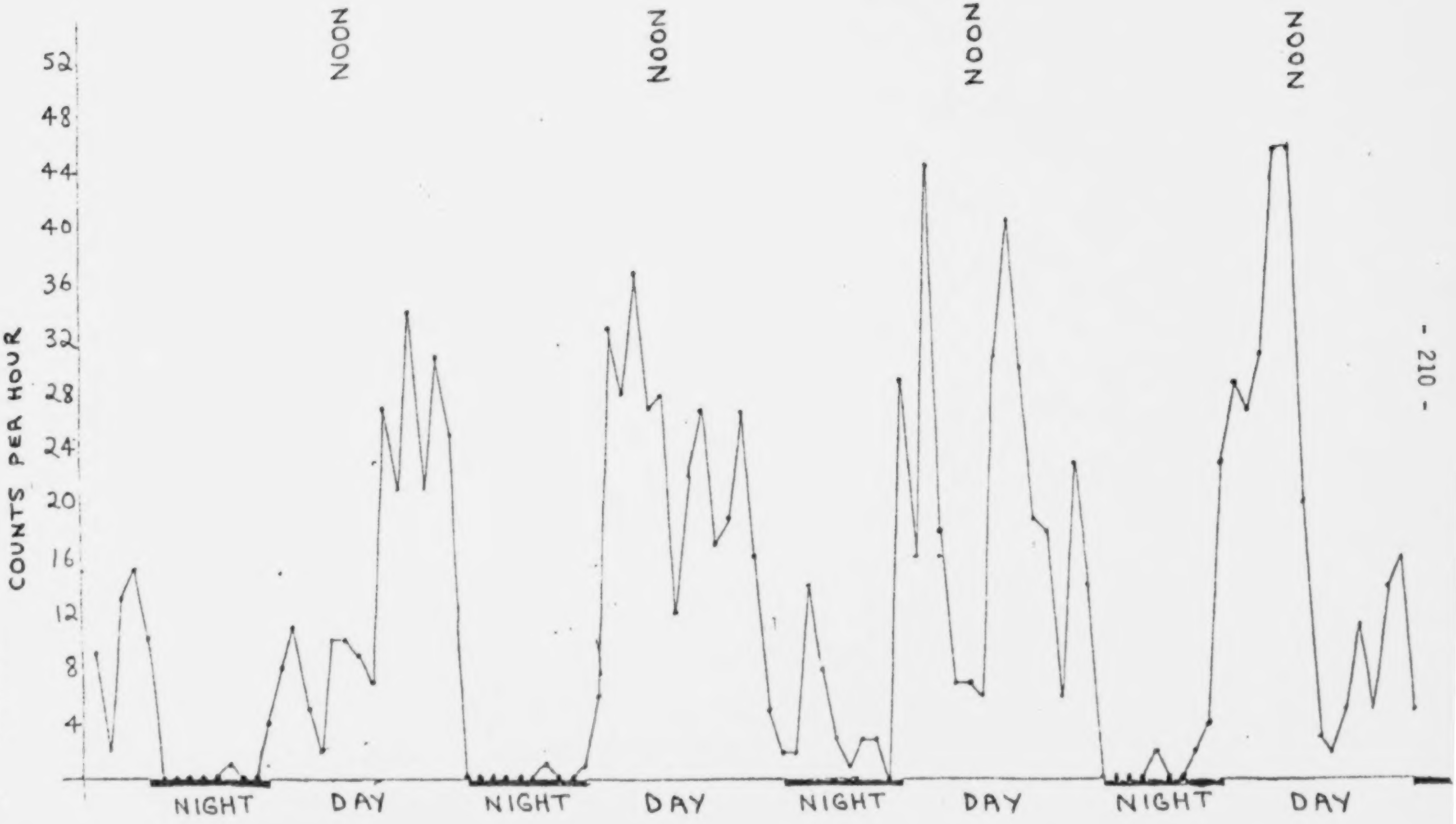

Figure 3. Examples of field model application. Manual plot of circadian activity pattern of caged yellow perch (Perca flavescens), in Heming Lake, Manitoba.

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ABSTRACT

The Environmental Protection Service's Aquatic Toxicology Laboratory in Edmonton has developed a recycle holding system design for the rearing and maintenance of fish stocks for use in toxicity testing. Three species of fish, rainbow trout (Salmo gairdneri), chum salmon (Oncorhynchus keta) and threespine stickleback (Gasterosteous aculeatus) have been successfully held in large numbers in both freshwater and artificial marine recycle systems.

This paper provides technical and bibliographical information for the construction and operation of these types of systems. Also, logistical and economical justifications for the use of these types of holding systems are given. Problems encountered and possible modifications to increase the flexibility of these systems are also discussed.

Finally, results of toxicity testing using fish held in these systems are given for various substances such as reference toxicants and industrial discharges. Where possible, these results are compared to toxicity data using fish held in conventional single-pass systems.

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INTRODUCTION

Life support systems for holding aquatic organisms can be divided into two general types: single-pass and recirculation. In a single-pass system the water that is to be used is continually treated to achieve acceptable parameters, pumped to the holding facility and then discarded. Depending on the original quality of the water (often chlorinated municipal tap water) and the quantity of water needed, this type of system can be very complex and expensive (see Appendix I).

In a recycle system the intent is to continually regenerate the holding water to acceptable parameter levels. Again, the original water may need pretreatment before addition to the system initially or as make-up water for losses due to evaporation or spillage.

Since 1974, the Aquatic Toxicology Laboratory (ATL) has been developing recycle systems in order to have the capability to perform marine toxicity testing at an inland location. This work has led to a design which is easily built and maintained and is also highly flexible. The purpose of this paper is to provide general information on the construction and use of a recycle system. Rather than report on areas of fish management that are well documented in the literature, it is the intent of this paper to describe techniques which have been learned by trial and error. It is hoped that subsequent experimentation will provide more detailed qualitative data about recirculation systems. A bibliography is appended for those who require more detailed information.

DESCRIPTION

A conventional single-pass water treatment system for freshwater organisms is presently in use at the ATL. Treatment of the water is as follows:

1. City of Edmonton water is brought into the laboratory and pressurized carbon dioxide is injected into the water to lower the pH from the range of 9.0-9.5 to the range of 7.0-8.0.

2. Solids (greater than 5 microns) are removed from the water by using an anthracite coal (Anthrafilt) filter.

3. An activated carbon filter is then used for chemical filtration, particularly chlorine removal.

4. The water then enters a 4500 litre polyethylene reservoir where aeration is supplied and temperature control is maintained. Also at this point a 312.5 mg/l solution of sodium thiosulphate is dripped into the reservoir at a rate of 40.0 ml/min. The resultant concentration in the laboratory treated water is <0.25 mg/l. This solution is added to the water to lower the total and free residual chlorine levels to <0.01 mg/l.

5. Ultraviolet sterilization is used for disinfection of the water and for chlorine removal. Disinfection is required because the 4500 litre reservoir is open and provides a potential access point for airborne bacteria and viruses.

6. The water is pumped throughout the laboratory and, after use, is discarded to waste.

This system requires a considerable capital expense plus several monitoring and recording devices to ensure a continual supply of acceptable treated water. Added to this are high operational costs for city water, carbon dioxide, filter media and other consumable items.

A recycle system designed to maintain water in a comparable condition to the laboratory treated water is shown schematically in Figure 1. One cycle of this system is as follows:

1. Water flows into the fish holding tank under low pressure creating a current and turbulence at the surface of the water. The current promotes swimming activity in the fish and flushes waste materials from the tank sides and bottom through the central standpipe.

2. The holding tank presently in use is a 1000 litre circular fiberglass tank of conventional design. The standpipe determines the water level in the tank. There should be threaded outside fittings at the drain that allows the drainage pipe to be attached securely.

3. The drainage pipe leads to a bulkhead fitting in the bottom of a 200 litre open-topped, polyethylene container. This is the settling tank that allows the majority of the solids generated in the holding tank to settle out and accumulate. These solids (faecal material and uneaten food) can then be periodically siphoned from the system. This tank also adds extra volume to the system and provides a convenient access point for the removal or addition of water. An overflow in this tank leads to the biological filter.

4. The biological filter is shown in Figure 2. The filter is constructed out of 3/4" plywood and its dimensions are 8' x 2.5' x 2'. Water entering the filter at a rate of about 10 l/min is evenly distributed over the surface through a series of slotted pipes. This water percolates through a one-inch substrate of oyster shells (The bacterial mechanisms that detoxify the water will be discussed in a following section.). The water then passes through a slotted fiberglass filter plate into the bottom half of the filter. A portion of this water is returned via airlifts to the surface and the process repeated. The remaining water is collected in a series of pipes at the bottom of the filter and pumped by an electric water pump to the carbon filter. This pump supplies the entire working pressure for the recycle system.

5. The carbon filter is a small fiberglass shell containing three cubic feet of activated carbon. Valves on the filter allow it to be backwashed as required. Water leaving the carbon filter is piped to the fish holding tank completing one cycle.

BIOLOGICAL FILTRATION

Spotte (1970) describes biological filtration as the mineralization, nitrification and denitrification of organic nitrogenous compounds by bacteria suspended in the water or attached to the gravel in the filter bed. Put more simply, bacteria that convert toxic metabolic by-products to less toxic forms are encouraged to grow on the substrate in a specially constructed filter. The basic nitrification cycle of waste products is

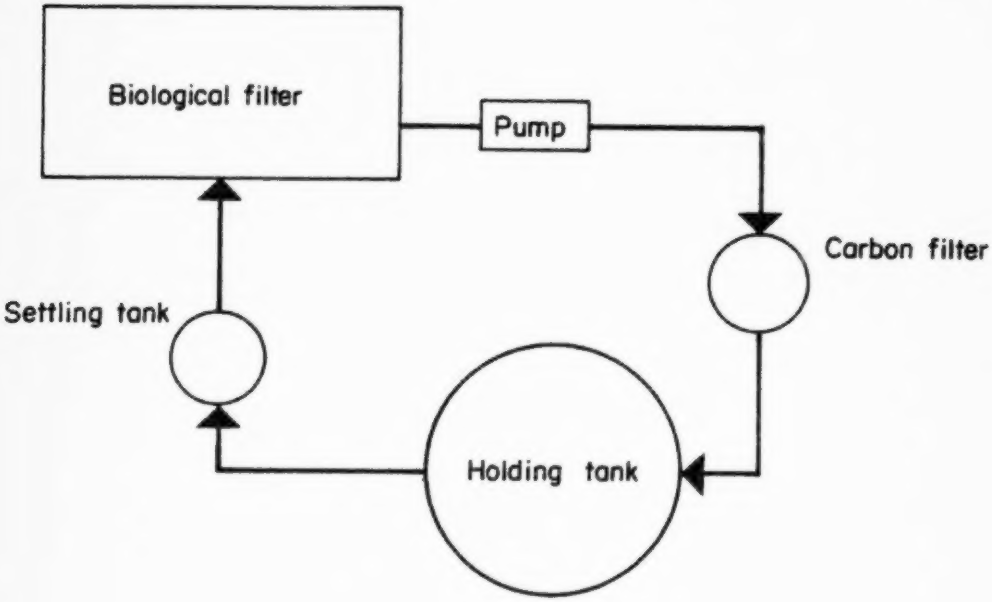
Biological filter
Pump
Carbon filter
Settling tank
Holding tank

Figure I. Schematic diagram of a recycle holding system.

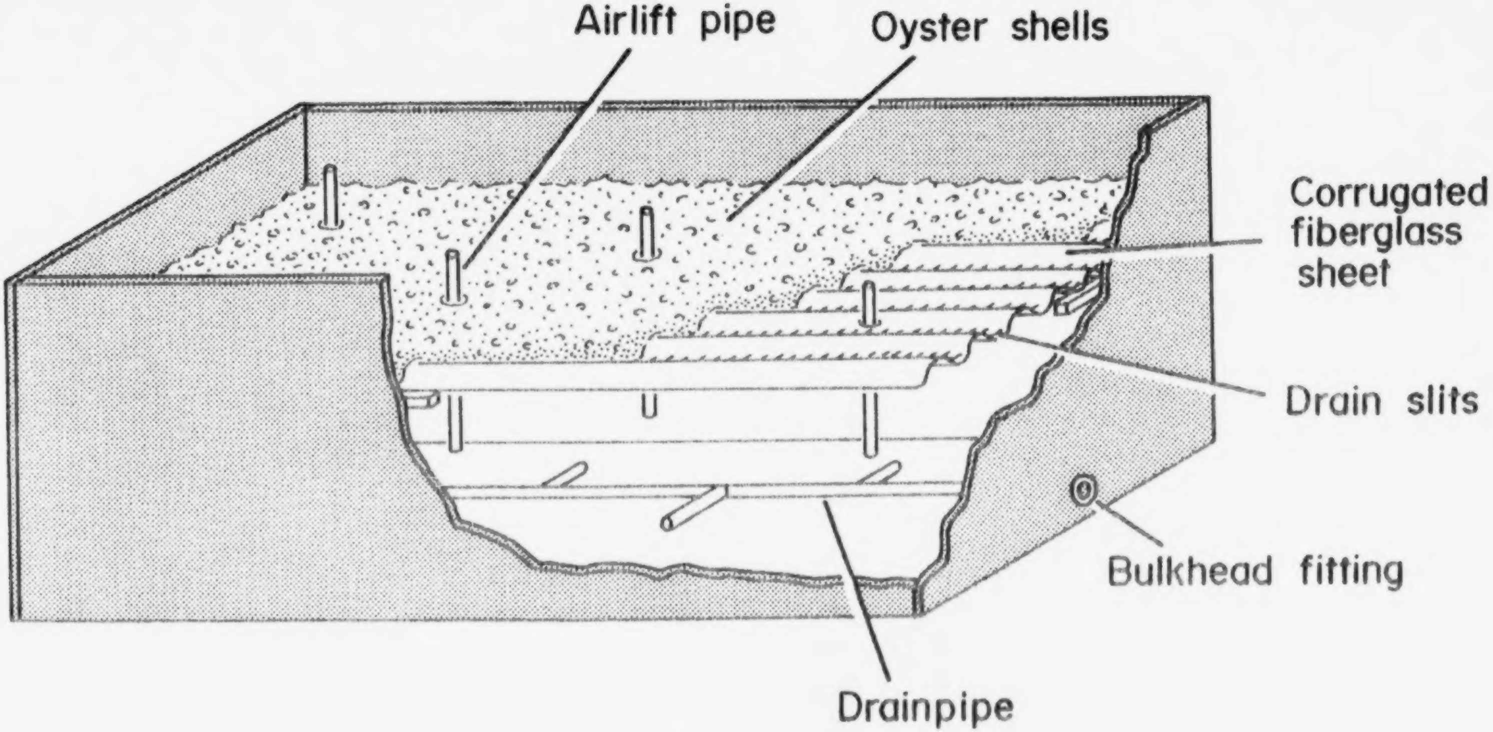
Airlift pipe
Oyster shells
Corrugated fiberglass sheet
Drain slits
Bulkhead fitting
Drainpipe

Figure 2. Biological filter.

from ammonia to nitrites and nitrites to nitrates. Denitrification converts nitrates to nitrous oxide or free nitrogen. Concurrently, the toxicity decreases with ammonia and nitrites being more toxic than nitrates. A review of the literature indicates that the lethal level of ammonia (NH_3) to rainbow trout is 0.2 mg/l, the 96 hour LC50 of nitrite nitrogen to rainbow trout is in the range of 0.19 to 0.39 mg/l and that the 96 hour LC50 of nitrate nitrogen to rainbow trout is 1360 mg/l (Quality Criteria for Water, 1976). The typical levels of these by-products in our recycle system are an ammonia (NH_3) concentration of <0.01 mg/l, a nitrite nitrogen concentration of <0.02 mg/l and a nitrate nitrogen concentration of 65.0 mg/l. A stable recycle system will have very low levels, if any, of ammonia and nitrites and higher levels of nitrates.

Under ideal conditions, denitrification would reduce the nitrate levels. However, this process occurs best under anaerobic conditions and is inhibited by oxygen concentrations greater than 1 mg/l (Wheaton, 1977). Spotte (1970) states that denitrification is also possible with aerobic bacteria and that anaerobic denitrifiers can occur in the biological filter bed. At present, there is no indication that any denitrification is occurring in the ATL's systems.

In order to control the nitrate concentrations, approximately 20% of the water in the system is periodically drained off and replaced with new water of similar quality to that used to start the system. This procedure is also recommended to reduce the levels of organic acids and other materials that accumulate in most recycle systems.

Readers requiring more detailed information on this process are directed to Spotte (1970) and Wheaton (1977) and the bibliography of this paper.

FILTER MEDIA

The filter media in the biological filter provides a large surface area of substrate for bacteria growth and performs as a coarse mechanical filter for solids removal. Another important function of the media is to buffer the pH of the water since recycled water tends to become acidic because of accumulating organic materials and the production of H+ ions during the nitrification process (Wheaton, 1977). The chemical composition of oyster shells provides a continual pH buffer. These shells are readily available at feed stores as poultry grit and work adequately. The angular shape and size (approximately 2 to 5 mm) of the ground shells provides a good substrate for the bacteria and a good drainage through the filter.

OPERATION

There are several factors about the operation of the biological filter that are important in determining the success of the bacterial culture. These are:

1. When starting up a new filter, there will be definite time lags between the accumulation of the various components of the nitrogen cycle and the growth of bacteria that convert them. During this period the fish in the system are subjected to toxic levels of these metabolites. This can be prevented by starting the system with only a small number of fish present. The population can then be slowly increased as the number of bac-

teria increase. This process can be hastened by dosing the system with unsterilized water or innoculating the filter with media from another bacterial population. Carmignani (1977) has shown that seeding a filter in this way can almost halve the length of time required to activate a filter.

2. The biological filter is a consumer of dissolved oxygen. If the filter does not receive sufficient oxygen, it will either "die" or will start anaerobic metabolism. The by-products of an anaerobic biological filter can be toxic plus there is no nitrification in the absence of oxygen.

3. Any changes in the filter environment such as pH, temperature or salinity can retard or even kill the bacterial population. Care must be taken to ensure a stable filter environment.

4. While a certain amount of detritus on the biological filter is desirable to support the bacteria and aid in mechanical filtration, an excess can lead to clogging, chanelling and/or suffocation of the filter. Excess material can be removed by siphoning or by skimming the media surface with a net. The gravel should not be excessively disturbed as this could affect the performance of the bacteria cultures and cause a subsequent rise in the ammonia and nitrite concentrations. Our biological filter is not constructed in a way to allow for backwashing of the filter media. Backwashing can remove much of the nitrification bacteria and reduce filter efficiency (Wheaton, 1977).

TEMPERATURE CONTROL

Temperature control in the laboratory is often the single most expensive problem encountered. If the water in a holding system needs to be warmer than ambient, electric heaters with thermostatic controls are sufficient. However, lowering and maintaining the holding temperature below ambient is more difficult.

In the case of the ATL, the entire recycle system is located inside a controlled environment chamber capable of maintaining a given temperature plus or minus 0.5°C. This room is a considerable capital expense and might not be practical in most circumstances.

An alternative would be to place refrigeration coils in the system itself. Depending on the available materials, the coils could be placed throughout the system or in a central place such as the fish holding tank or the settling tank.

MARINE ADAPTATIONS

Up to this point the discussion of the recycle system has been the same for freshwater and marine conditions. The two major concerns of running a recycle system with saltwater are the source of the water and problems associated with corrosion.

The ideal source for marine water for a system would be the ocean. However, for inland locations collection and transport would be extremely expensive. The only viable alternative is to use an artificial marine mix. Recipes are available for the mixing of artificial marine water (Spotte, 1970; Clark and Clark, 1964; and Standard Methods, 1975). There are good commercial products available that are also acceptable (Most inland aquariums use a commercial mix for marine water.). At present there is little information available on comparisons of artificial and natural marine waters with reference to their ability to support marine life. However, Gallagher and Brown (1976) have shown that artificial marine mix is a suitable medium for culturing juvenile lobsters and may even result in better overall growth.

Marine water is highly corrosive and all metal surfaces in or near the system must be protected by paint or otherwise sealed. All electrical connections should be protected and made waterproof and electrical circuits should have ground-fault interrupters.

MODIFICATIONS AND FUTURE PLANS

This type of system lends itself to modifications required by individual circumstances. A variation of the single holding tank system has been run successfully at the ATL for several years (see Figure 3). The major changes for this system include the addition of a 4500 litre reservoir, an ultraviolet sterilizer and three more fish holding tanks. The reservoir increases the lifetime of the system by increasing the ratio of water to fish weight. The holding tanks are elevated and connected to a common drainage pipe which leads to the settling tank. Because of the open reservoir, it is not possible for the pump at the biological filter to supply the entire system with pressure. If there is sufficient water head available a simple siphon or gravity feed system could be used. A second pump in the reservoir could also provide the flow to the holding tanks. In this case, to control the water level in the biological filter and in the reservoir, it is necessary to control both of the pumps with a system of floats and switches. This ensures that neither point in the system can overflow or be pumped dry. If both pumps have the same output, they will tend to cycle on and off due to the smaller volume in the biological filter. This intermittent flow is undesirable in the fish holding tanks due to the possibility of stressing the fish and can be prevented by undersizing the pump in the reservoir. This pump will run continually while the pump in the biological filter will cycle on and off. There is a small return pipe to the reservoir at the highest point in the reservoir pump pipe system. Without this pipe the water in the system could siphon even with the pump turned off. This pipe bleeds air into the system when the pump is off and breaks the siphon.

The ultraviolet sterilizer is included in the system just after the biological filter. Shelbourne (1964) and Spotte (1970) state that bacteria control is desirable in closed systems due to the high levels of organic materials that can occur. Shelbourne (1964) provides a design for a relatively inexpensive ultraviolet sterilizer that can be constructed from plywood. This provides an alternative to the high pressure models available commercially.

As with most prototype systems, the design has several areas where the operation and/or maintenance could be made more efficient. These include:

1. Even with crushed oyster shells providing a buffer the tendency in a closed system is towards acidic conditions. Possible solutions to this problem are:

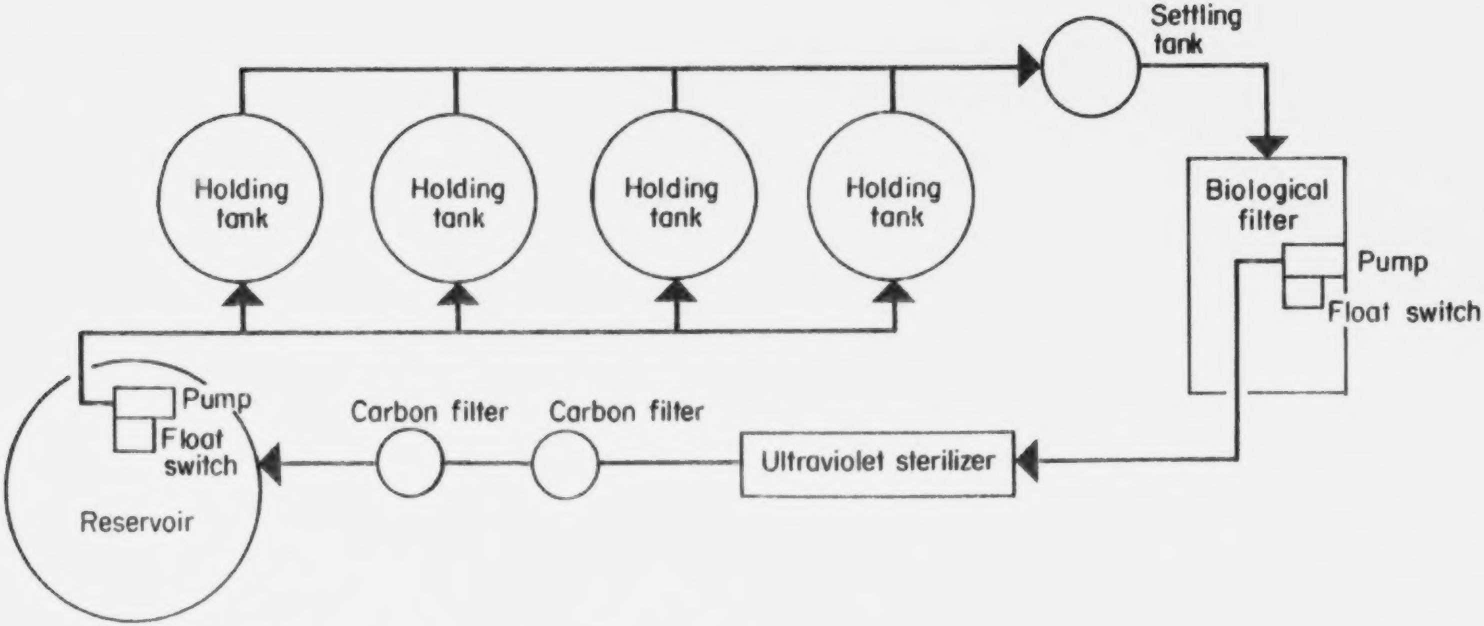
Settling tank
Holding tank
Holding tank
Holding tank
Holding tank
Biological filter
Pump
Float switch
Pump
Float switch
Carbon filter
Carbon filter
Ultraviolet sterilizer
Reservoir

Figure 3. Schematic diagram of an expanded recycle holding system. The float switches control the pumps to maintain water levels.

a. increase the volume of oyster shells in the biological filter to a thickness of 2-3 inches,
b. determine if organic materials are coating the shell granules, and
c. add other calcareous agents such as marble chips or limestone to the system to increase the buffering capability.

Siddall (1974) proposed that acid-soluble, non-organic materials coated the substrate and reduced the buffering activity. Dry tumbling of the filter media restored the buffering ability of the material.

2. Faecal and food wastes can clog the biological filter under heavy biological loadings. Possible countermeasures include the use of screens or other solids removal devices and/or filter backwashing to a limited extent.

3. At present, pump control is provided with floats and microswitches. These devices are relatively inexpensive but are subject to corrosion and other failures. They should be replaced by solid state level controls.

4. At present, three species of fish have been successfully maintained in these systems. Future plans include the holding of other species of fish and also the breeding and hatching of laboratory species (*Gasterosteous aculeatus* and *Salmo gairdneri*) in order to test the limits of the systems. Another area of interest is the culture of various freshwater and marine molluscs and crustaceans.

5. As stated before, little information on the validity of toxicity testing using recycled waters is available. In the following section, the results of preliminary testing will be given. Future plans include toxicity testing of chemicals and effluents to compare the responses of organisms acclimated to single pass and recycle systems. Comparisons of the responses of fish acclimated to real and artificial marine waters will also be carried out.

6. Recycle systems tend to accumulate nitrates. Various authors (Shelbourne, 1964; Siddall, 1974; and Wheaton, 1977) have described systems utilizing plants to remove these nitrates. At the ATL, *Lemna* sp. and *Fucus* sp. have been used in the freshwater and marine systems respectively. Preliminary results show that these plants are working to reduce the nitrate concentrations.

7. It is planned to submit samples of recycled waters for detailed chemical analysis in order to determine long term changes in the water quality.

TOXICITY TESTING

Procedures for toxicity testing using organisms held in recycle systems are similar to the ones used in single-pass systems. There are, however, several problems resulting from the recycle aspect. The main problem encountered is that bioassays should be run using the holding water as the diluent for all test concentrations plus the control water. In a single-pass system, there is a continual supply of holding water, while under recycle conditions, the water supply is finite. To provide dilution and control water of the same quality as the holding water, the water must be removed from the holding system. This water is then replaced with the same quality water used when starting the system. Replacement water can be prepared in a batch process. Depending on the water requirements for setting up a bioassay, a single-pass system could prove more practical. In our freshwater recycle system, approximately 4000 litres per day can be removed at one time for use as diluent or control water. In our marine recycle system, only 360 litres/day can be removed.

One consideration of toxicity testing in recycled waters is the effect that the water quality will have on the observed response. Recycled waters develop high nitrate levels and at present, little information is available on this problem.

The recycle system does lend itself nicely where toxicity is to be observed under carefully controlled conditions. It is possible to artificially manipulate the water quality in the holding system. For example, it is well documented that heavy metal toxicity is dependent on the hardness of the water. In a single-pass system it would be very difficult to continually maintain an artificial water hardness concentration. In a recycle system this could be done initially and then only as required for replacement water. At any time it would be possible to change the hardness level as desired.

Many toxicity tests have been performed at the ATL using fish held in either freshwater or marine recycle systems. At present, rainbow trout, chum salmon and threespine stickleback have been used as test organisms. Because the ATL is a pollution monitoring laboratory, the majority of the tests are performed using industrial effluent discharges. Although comparisons are not always possible due to the uniqueness of each sample the results have been consistent with those shown in similar testing (Moore et al., 1976). The results for the drilling mud discharge of an Arctic operation are shown in Table 1. In all cases no control mortalities were observed.

TABLE 1: 96 hour LC50 values determined for drilling mud from an Arctic operation. The test species was *Gasterosteus aculeatus* acclimated to a salinity of 25 parts per thousand in a recycle system.

DEPTH (feet)	96 HOUR LC50 RANGE (%)
2554	4 to 10
5031	1 to 4
7601	1 to 5
10008	< 2
12500	2 to 4
15774	2 to 4

Some preliminary work using reference toxicants has been performed at the ATL. The results of testing with phenol using threespine stickleback acclimated to a salinity of 25 parts per thousand gave 96 hour LC50's in the range of 5 to 10 milligrams per litre. EVS (1977) reported values of 1.3 to 1.4 milligrams per litre under similar conditions but with natural seawater in a single-pass system. These results, although relatively close, do indicate that further investigative work must be performed in this area to determine the validity of comparing results using single-pass and recycle systems.

CONCLUSIONS

The recycle system is simple, inexpensive to operate and can be located almost anywhere. Hopefully, it will provide a viable alternative to industries, private companies and government agencies for whom comprehensive toxicity testing has not been practical.

In the future, the primary area of investigation in recycle systems at the ATL will be the verification of the results of toxicity testing. This will necessitate a great deal of comparison testing at the laboratory and the co-operation of freshwater and marine testing facilities across the country. The ATL also hopes to develop culture techniques for other freshwater and marine organisms. This will result in a broader view of the effects of toxic substances on the aquatic environment.

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APPENDIX I

THEORETICAL COST OUTLINES FOR TWO COMPARABLE AQUATIC LIFE SUPPORT SYSTEMS

The following are approximate costs for setting up two life support systems, one single-pass and the other recycle. The single-pass system is that described as presently in operation at the ATL and the recycle system is the one shown in Figure 3. In order to properly compare the cost of each system, it was decided to determine the approximate expense in setting up a facility for holding 2000 rainbow trout for a twelve month period. The following assumptions have been made:

1. All costs listed are approximate.

2. Expenses that would be incurred for both systems equally are not considered. These include building rent, labour, equipment and tools, fish tanks etc.

3. The single-pass system has an actual capacity of approximately 1.5 times that required for this hypothetical situation. Consequently, the final total cost is reduced accordingly.

SINGLE-PASS SYSTEM	
Carbon Filter	4335.00
3 Changes of Carbon	996.00
Anthrafilt Filter	4185.00
2 Changes of Anthrafilt	500.00
Sodium Thiosulphate System	50.00
CO_2 (pH Control)	1150.00
Main Pump	270.00
pH Monitor[1]	3000.00
Chlorine Monitor[1]	3700.00
Security System[1]	1476.00
Refrigeration Unit	3000.00
Water (approximately 5×10^6 IG)	10000.00
Sub-Total	32662.00
Less 33%	10778.46
TOTAL	$21883.54

RECYCLE SYSTEM	
Biological Filter[2]	100.
Oyster Shells	12.
Pumps[3]	320.
Microswitches	50.
Carbon Filter[4]	1500.
Environment Room[5]	8546.
Water (3000IG plus 20% make-up per month) = 10200 gallons	20.
TOTAL	$10548.

NOTES

1. Water quality monitoring not considered necessary to recycle system.

2. Constructed by laboratory staff using simple methods and standard materials.

3. Submersible pumps that could be replaced by less expensive non-submersible pumps.

4. High pressure fibreglass body. This filter could be replaced by a "home-made" version as outlined in Spotte (1970).

5. Environment room could be replaced by less expensive refrigeration units.

The above cost outlines indicate an approximate saving of 50% for the recycle system. The main area where costs are lowered is the enormous reduction in the quantities of water consumed by each system. It is estimated from the above figures that the recycle system would use approximately 0.3% of the water consumed in the comparable single-pass system. The costs in preparing the water for the recycle system are assumed to be negligible. Since the time required to produce the desired water quality is not critical, inexpensive methods may be used. Dechlorination of the water can be performed passively by allowing the water to stand, however, this method does not remove chloramines if they are a problem. Costs are also significantly reduced by the lower overall capital expenditures required for the recycle system.

It should be noted that the final expense given for the recycle system is not necessarily the actual cost that would be encountered when setting up such a system in the beginning. As outlined in the notes, several parts of the system could be modified or replaced with less expensive equipment. Approximately 80% of the cost outlined for the recycle system is due to the high capital outlay for the controlled environment chamber.

Mayfield*, C.I., W.E. Inniss*, and J.E. Thompson*. 1979. Bioassay techniques for toxicological studies on aquatic organisms. Proc. Fifth Annual Aquatic Toxicity Workshop, Hamilton, Ontario, Nov. 7-9, 1978. Fish. Mar. Serv. Tech. Rep. 862, pp. 225-231.

ABSTRACT

Two techniques for examining the effects of toxic materials on members of the aquatic biota are described. The first involves u.v. fluorescence microscopy of sediment bacteria in incubation chambers after staining with 8-anilino-1-naphthalene sulfonic acid. Periodic examination of the same sediment sample allows the effects of added toxicants on the sediment microorganisms to be assessed. The second technique uses the phase transition temperature of membranes from algae as an indicator of damage caused by the application of toxic materials. Increases in this transition temperature after treatment of algae with toxic materials were observed.

Key words: Bioassay, sediment bacteria, fluorescence staining, membranes, transition temperature.

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INTRODUCTION

This report describes the details of two methods developed to assess the effects of toxic materials on members of aquatic ecosystems. The first method examines the effects of such materials on the heterotrophic bacteria in sediments, using sediment samples incubated under controlled conditions in the laboratory and examined by direct fluorescence microscopy. The second method was used to examine the effects of toxic materials on specific members of the algal microflora; the physical state of membranes of the organisms was used to estimate the "damage" caused by application of toxic materials.

Methods

1. Direct fluorescence microscopy.

Direct observation of sediment bacteria usually entails staining with a fluorescent dye such as acridine orange, washing to remove excess dye and observation with incident u.v. or blue light. Most dyes used are toxic to the microorganisms in sediment, and the washing procedures used mean that the relationship of the bacteria and other microorganisms to the sediment materials is disturbed. In previous studies (Mayfield, 1975; 1977), staining of soil samples with a fluorescent probe (the magnesium salt of 8-anilino-1-naphthalene sulfonic acid) has been suggested as a way of examining soil samples so that the disturbance of such samples is minimized and the distribution of microorganisms in the soil could be determined. This was possible since this type of fluorescent probe changes the wavelength of light emitted upon u.v. or blue light excitation depending upon the molecular environment. For example, proteins exhibit a peak emission at 470 nm when excited in aqueous solution and so washing procedures are not required. In the present study, another fluorescent probe, the ammonium salt of 8-anilino-1-naphthalene sulfonic acid, was used as the staining agent. Other members of this group of fluorescent probes, such as the free acid and the sodium or magnesium salts, also have the same properties and may be used with different sediment types if they prove more suitable under particular conditions.

Sediment cores were used as a source of subsamples which were removed and placed in perfusion chambers similar to those designed for soil studies (Polonenko et al., 1978). Each unit contained three separate chambers in each of three compartments. Each chamber was 24 mm x 24 mm x 4 mm deep. The overall dimensions of the perfusion apparatus were 88 mm x 88 mm so that they were able to fit onto the stage of a microscope. The slides were prepared for incubation as outlined in Polonenko et al., (1978), with the outer chambers containing the nutrient solutions and the test materials and the inner chambers containing the sediment samples. Thus each unit contained three sediment samples flanked by two chambers containing the nutrients and test solutions. The staining solution was 2.5 mg ml^{-1} of 8-anilino-1-naphthalene sulfonic acid in distilled water and was added to the outer chambers for 4 h before microscopic examination. The staining solution percolated into the sediment samples and stained the microorganisms. This process occurred through the glass fibre strips connecting the outer chambers with the inner chambers containing the sediment. A Nikon Apophot microscope equipped for incident illumination was used and the filter combinations were the violet excitation, the violet dichroic mirror and the violet eyepiece barrier filter system on the microscope. An HBO200 mercury vapour lamp was the source of illumination. When the sediment was covered with a cover slip and examined with this epiillumination system, the bacteria and other microorganisms fluoresced strongly, and only a slight degree of background fluorescence was observed. Algal cells in the sediment samples showed typical red fluorescence due to chlorophyll. The slides containing the sediment samples were then incubated at 20°C, and the process was repeated. The slides maintained the moisture levels because of the glass fibre strips used to transfer liquids to the central compartments containing the sediment samples. Any material placed in these outer chambers also was transferred to the inner compartments. Each sediment sample was maintained in a water-saturated state throughout the period of the experiment. The observations were repeated at intervals up to a total incubation time of 35 days. The number of microorganisms in random microscope fields from the sediment samples was determined either by direct counting or from photographic records of the fields.

The technique has many different potential applications. The effects of nutrients on the development of sediment microorganisms may be examined, the effects of single or multiple doses of toxic materials on the system may be determined, the effects of doses of mixed toxic compounds may be examined for either additive or synergistic effects, the effects of different temperatures, oxygen tensions, pH levels, etc., on the results of toxic material application may be examined, and the colonization of solid materials in sediment samples may be followed directly with this method. Further applications include examination of the transformation of materials in sediments by percolating them from one chamber to the other, through the sediment sample. In this way, both the response of the sediment microorganisms to the material, and the changes in the material, can be determined.

2. The physical state of membranes in algae.

Membranes are in the main composed of protein and phospholipid. The phospholipid is arranged in a fluid bimolecular leaflet with the polar head groups oriented outwards and the fatty acid side chains projecting inwards into the membrane interior. Those membrane proteins termed integral proteins are amphipathic in nature which means that they have both polar and nonpolar portions. These proteins are arranged in the membrane in such a manner that their nonpolar portions penetrate into the membrane interior to interact hydrophobically with the fatty acid sidechains of the phospholipids, whilst the polar moieties remain in the polar environment on the outside surfaces of the lipid bilayer (Singer, 1974).

The lipid matrix of membranes is normally in a fluid (liquid-crystalline) state which means that the integral proteins are able to move through the plane of the membrane by simply diffusing through the lipid. However, if the temperature is lowered sufficiently, portions of the membrane lipid transform into a crystalline or gel phase in which the hydrocarbon chains form a rigid hexagonal lattice. The temperature at which this change first occurs is termed the lipid phase transition temperature, and it marks the beginning of the transition from the liquid-crystalline phase to the gel phase. These two phases of lipid can be detected by wide angle X-ray diffraction, and thus the lipid phase transition temperature can be determined (Engelman, 1970; Esfahani _et al_., 1971; McKersie and Thompson, 1977; Thompson _et al_., 1978).

Recently we have established by this technique that as cells deteriorate, increasing proportions of the membrane lipids become crystalline at physiological temperatures. This can be scored as an increase in the membrane transition temperature, and serves as a sensitive bioassay for cell deterioration attributable either to natural aging or pollutant-induced damage. For example, the data in Fig. 1 demonstrate that the transition temperature of smooth microsomal membranes from senescing cultures of Scenedesmus quadricauda increases with culture age from a low of 0°C for young cultures to a high of about 70°C for 140-day old cultures. This indicates that for young cultures the membrane lipid is entirely liquid-crystalline (fluid) at physiological temperatures, but as the cultures age, portions of the lipid become crystalline. Moreover, this increase in transition temperature shows a close temporal correlation with the loss of chlorophyll and loss of protein per cell, and can thus be construed to be an index of algal cell deterioration (Thompson et al., 1978).

This rise in transition temperature of microsomes with age signifies a lesion at the level of membranes in the senescing cells. For most biological membranes the phase transition temperature of the lipid is below physiological temperature, meaning that the lipid matrix of the membrane is in a liquid-crystalline phase (fluid). However, as the algal cultures age, the transition temperature rises well above physiological temperature, signifying that portions of the membrane lipid have transformed into the gel phase (crystalline). Loss of intracellular compartmentalization, which would be a consequence of increased membrane permeability, is an inherent feature of advanced algal senescence, and the presence of crystalline lipid in the early stages of senescence could account for these changes in permeability by engendering discontinuities in the bilayer where it interfaces with the adjacent fluid lipid (Thompson et al., 1978).

As cell deterioration advances, the proportion of membrane lipid in the gel phase increases. Wide angle X-ray diffraction patterns recorded at room temperature for smooth microsomes isolated from a young (29-day old) culture of Scenedesmus quadricauda feature two broad bands centered at Bragg spacings of 4.6 Å and about 10 Å. The 10 Å band is thought to be derived from protein but is not well characterized (Esfahani et al., 1971; Finean et al., 1968). The broad band at 4.6 Å is known to be derived from lipid in the liquid-crystalline phase (McKersie and Thompson, 1977; Thompson et al., 1978) and indicates that for young membrane the lipid is entirely fluid. For older cultures, algal populations can be separated by density gradient centrifugation on a layered sucrose gradient with three layers of 1.0M, 1.5M, and 2.0M sucrose. A layer of pink material remains on the surface of this gradient after centrifugation and was found to consist mainly of free membranes, which are presumably remnants of extensively senescent cells that have completely broken down. Wide angle X-ray diffraction patterns of this layer recorded at room temperature featured a sharp intense ring centered at a Bragg spacing of 4.2 Å, which is known to derive from gel phase lipid (McKersie and Thompson, 1977; Thompson et al., 1978). Virtually all of the lipid in the pink layer is in the crystalline or gel phase since the broad diffuse ring at a spacing of 4.6 Å (reflecting fluid lipid) was virtually absent. Thus, transition temperature, which in effect reflects the temporal progress of gel phase lipid formation (Fig. 1), can be used to score the age of laboratory cultures of algae and can also be used on algal blooms in the field. It can also be used to score the ages of different populations of algae that have been separated by density gradient centrifugation in the same manner as the separation of different types of cells of Scenedesmus quadricauda in the experiments described above.

Transition temperature can also be used as a bioassay method to score the degree of cell deterioration resulting from exposure to toxic materials. For example, wide angle X-ray diffraction patterns recorded at -30°C for membranes of a crude chloroplast fraction isolated from a plankton net sample taken from the Bay of Quinte, Lake Ontario, in August 1978, show only the broad band typical of liquid-crystalline lipid and centered at the 4.6 Å Bragg spacing, indicating that the membranes of these samples have lipid entirely in the liquid-crystalline phase. The algal bloom was just starting in the Bay of Quinte at the time of sampling. Preliminary experiments have indicated that when the algal samples are treated with as little as 1 ppm pentachlorophenol for 2 days, crystalline lipid is detectable at 5°C. Thus the transition temperature had risen from -30°C to a point above 5°C as a result of the treatment with 1 ppm pentachlorophenol.

Thus, change in transition temperature appears to be a response with high sensitivity that can be calibrated with the degree of cell deterioration, and therefore used to score for both lethal and sublethal effects of toxic materials. This potential for calibration arises from the fact that the transition temperature of the membrane lipid rises in an essentially linear fashion as deterioration progresses (Fig. 1), and thus serves as an index of the degree of deterioration.

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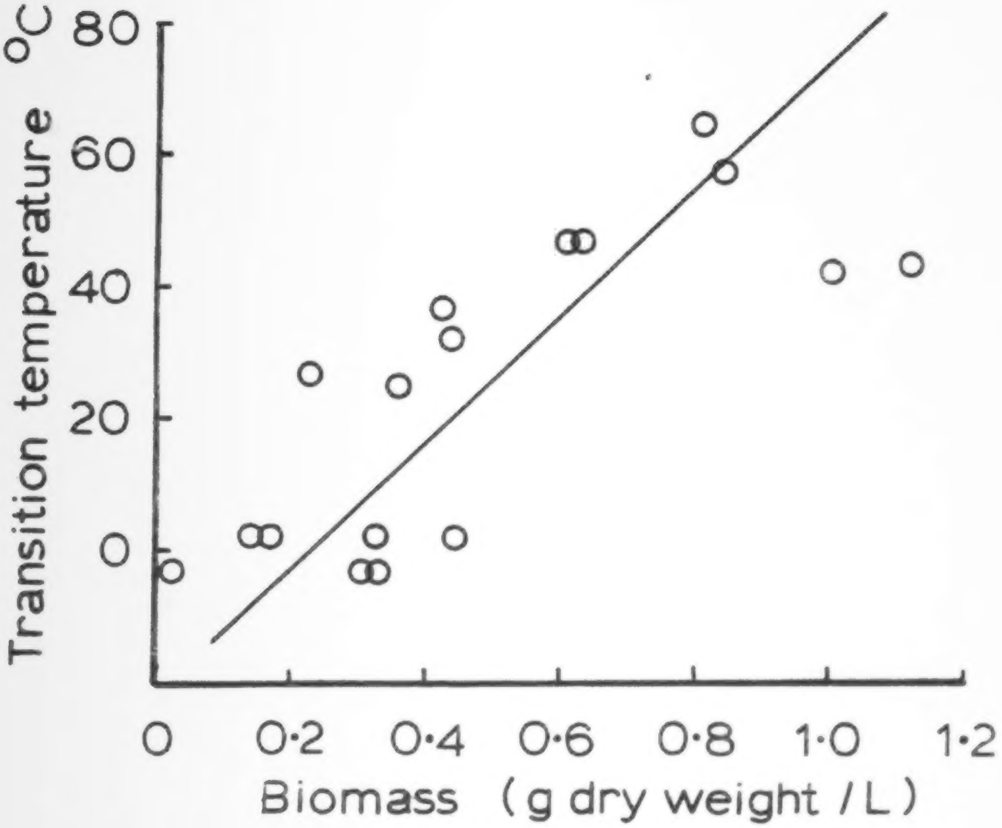

Fig. 1. Changes during senescence in the lipid transition temperature for smooth microsomal membranes from Scenedesmus quadricauda. Each data point comes from algal cultures of different ages, and therefore biomass per litre, grown in different carboys. (from Thompson et al., 1978).

Morgan II*, R.P. 1979. Biochemical parameters as pollution indicators in fishes. Proc. Fifth Annual Aquatic Toxicity Workshop, Hamilton, Ontario, Nov. 7-9, 1978. Fish. Mar. Serv. Tech. Rep. 862, pp. 232-240.

ABSTRACT

A variety of biochemical techniques have been utilized in an attempt to determine the overall impact of mixed and specific pollutants on fishes. The utility of biochemical parameters as pollution indicators in fishes with special reference to applicability, feasibility, specificity, and sensitivity is summarized from the currently available literature.

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In recent years, a great deal of interest has centered on the applicability of using biochemical parameters as indicators of pollutional stress in fishes. The catalyst for this interest first came from the accumulating information collected from laboratory bioassays and experiments indicating that stresses inflicted on test organisms may manifest themselves as sublethal effects, primarily of biochemical etiology. Second, acute effects of pollution in nature were rarely observed, but sublethal responses to pollution were present in a variety of aquatic organisms.

Initially, the intent of this paper was to summarize the utility of biochemical indicators in fishes for in vivo analysis of pollution with special reference to the applicability, feasibility, specificity and sensitivity of using these indicators in the field. Since we know, through primarily laboratory studies, that sublethal effects of pollutants occur at the biochemical level of organization, can biochemical monitoring be used to assay the overall health of an ecosystem especially in relation to point sources, but also to non-point source pollution? In essence, have we reached the point where state-of-the-art biochemical monitoring of the environment is feasible?

Monitoring of the aquatic environment is vitally important in two respects. First, biochemical monitoring of fish populations may be used as an early-warning system for the early detection of sublethal pollutional stress and for the assessment of the overall health of fish communities and populations. Second, biochemical monitoring of fishes serves as a tool in assessing the general effectiveness of specific water quality criteria.

A review of the available literature (which consisted of approximately 350 papers) may be best described as futile in assessing the overall utility of measuring biochemical parameters. Instead of futile, a better word may be "disjunct" since throughout the literature, there were few common threads of utility woven through the biochemical approaches investigated. It appears that some of the problems in the literature included an academic approach to biochemical testing. This academic approach may be a reflection of each investigation's fetish and pedantic passion for a particular enzyme or set of physiological-biochemical characters and is perhaps a reflection of previous work done with either the organism or the pollutant. Another glaring problem concerned the lack of both technique standardization and technique development for poikothermic animals such as fishes. In some cases, biochemical techniques for mammals may be used for fishes, but the majority of techniques requires an optimization of test parameters specific for the species in use during the study.

A major problem concerns the biochemical testing of a variety of species at different life stages. It is difficult to compare results for these tests when different pollutants, different protocols, different species and different life stages were used in studies assessing pollutional stress on an organism. Also, in view of the above set of problems, many studies appear to be instantaneous and are not concerned about the long-term patterns of the biochemical parameters. Finally, there appears to be a problem in quality control and lack of adequate sample size in many studies. In 53 of the studies where adequate statistical information was available, coefficients of variations, for the same test, differed by as much as 72.3% and as little as 6.4%.

With all of the above caveats in mind, I have changed the scope of this paper slightly in an overall attempt to review the use of biochemical parameters as monitoring aids with some discussion of the utility of these types of measurement.

Before a discussion on biochemical parameters can be initiated, it is necessary to develop some of the rationale associated with the current philosophy on the use of biochemical parameters as pollution indicators in fishes for the detection and monitoring of environmental stresses. In the environment or ecosystem of concern, increasing levels of a pollutant may initially cause some biostimulation, then a no-effect level followed by the occurrence of chronic or sublethal effects and finally, if enough of the pollutant is present, acute effects. Obviously, the area of concern is the set of effective concentrations of a pollutant between the no-effect level (Dinman 1972) and the level of pollutant causing acute effects at either species, population, community, or ecosystem level of organization. Typically, these effects are described as sublethal or chronic effects.

Responses to an environmental stress may be observed as either histological, morphological, physiological or ethological, or some combination of responses (Sprague 1971). No matter what the response is, the underlying stimulus or effector is typically an involvement of the pollutant and a biochemical pathway. The involvement of a specific pollutant with general cellular functions may cause several responses (Ariens et al. 1976) including anesthesia, interference with neurotransmission, disturbance of DNA-RNA synthesis, inhibition of cell division, suppression of the immune response, and mutagenic or carcinogenic responses.

One of the major problems observed in the literature was the lack of a strategic approach to determining the biochemical effect of a pollutant on the system level. Generally, most investigators were concerned with the effect of a pollutant, such as the effect of a pesticide on brain enzyme activity, in a specific physiological system. Usually, no attempt was made to see if other physiological systems were also influenced by the pollutant of interest. A variety of systems (biliary, renal, digestive, hematological, endocrine, exocrine, reproductive, immunological, neurological, respiratory, and hepatic) occur in fishes and it appears that for certain pollutants, responses occur in more than one system (Morgan et al. 1973).

Generally, the effect of a pollutant, observed at a system level, is a function of the biochemical effect of the pollutant at the cellular level. For example, the pollutant may bind with proteins (especially albumin), change the properties of the cell membrane, modify enzyme activity, inhibit or modify enzyme - protein synthesis, disrupt oxidative phosphorylation, and affect non-enzymatic or proteinous material. These reactions, at the biochemical level, dictate the responses at higher levels to the specific pollutants. Therefore, in any long-term evaluation of pollutant toxicity, the underlying biochemical reaction is of fundamental importance rather than a complete understanding of the response at higher levels.

To be useful in any type of monitoring work, the biochemical parameter (or set of parameters) should have three attributes. It should be sensitive, specific and sensible. In almost all of the surveyed literature, few attempts were made to characterize the biochemical parameter investigated as to its ultimate sensitivity in response to low levels of pollutants. Some parameters appeared to be sensitive to aquatic pollutants in the μg/liter range (Hodson 1976; Christensen et al. 1972, 1977) or ng/liter range (Johnson 1968) while others are sensitive at mg/liter levels (Jackim et al. 1970). The question of sensitivity is also relevant to experimental design. A major weakness in the literature appeared to be lack of preliminary experiments (at least, it wasn't stated as such) with a pollutant to determine the sensitivity of the biochemical parameter as far as degree of response to a pollutant. It is an obvious advantage, for monitoring purposes, to have a biochemical indicator which is extremely sensitive to low levels of a pollutant. The majority of reported tests in the literature do not fit this category.

Specificity in a biochemical parameter is desirous, although there are only limited examples in the literature. However, examples do include acetylocholinesterase inhibition by pesticides (Coppage et al. 1975), induction of specific enzymes through mixed function oxidase systems (Payne 1976, 1977; Kurelec et al. 1977; and Stegeman 1978), and inhibition of δ-amino levulinic acid dehydratase (Hodson 1976; Hodson et al. 1977). The number of apparent specific biochemical responses to a pollutant appears to be increasing as more research interest is directed in the area. Of particular interest are the array of inductible enzymes (Payne 1976, 1977) in estuarine and marine organisms.

The attribute of specificity in a biochemical response to stress is obviously attractive. Yet, non-specific or generalized responses of a biochemical nature to low-level pollutants may also be useful in indicating stress. Although biochemical responses may not be specific and usable in characterizing a pollutant, the fact that they could indicate unfavorable conditions is important in itself.

The last desired attribute of a biochemical parameter is sensibility. A sensibility factor should be considered with all proposed biochemical monitoring. Some types of tests require rather elaborate facilities associated with biochemical-physiological laboratories, but not with many routine analytical facilities. Sensibility needs to be considered if there are sample collection problems in the field such as extra handling or preservation techniques. An example of a routine test that may not be feasible is GC-MS analysis (due to cost and sample handling, and preparation).

Another problem in the use of biochemical parameters in the field as pollution indicators is the identification or discrimination of abnormal individuals in an array of sample values. Obviously, a large amount of noise in the system, due either to the variability of the biochemical parameter within a population or to analytical variability, will create problems in interpretation. Consequently, it is first a necessity, in order to isolate the abnormal values, to establish a normal data base. A data base for the biochemical parameter of interest, first of all, allows an investigator to identify the abnormal or stressed organism and to follow temporally and spatially the occurrence of these abnormal individuals in the population or populations of interest. Also, a set of normal values is required in order to estimate the variability within the unstressed members and within the stressed members of the population. Finally, measurement of the baseline allows some estimate reliability among laboratories, sampling periods, etc. Very few of the papers in the literature provided any estimate of the reliability or variation within a biochemical parameter.

Throughout most of the literature relating to bioassay work, the majority of investigators were always comparing the responses of stressed animals to those of control animals presumably handled similarly in a framework of good experimental design, randomization, etc. For work _in vivo_, the preceding approach will not work. For _in vivo_ analysis of pollution effects as monitored by biochemical responses, the baseline parameters may need to consider diel, lunar, seasonal, reproductive, yearly, age and sex effects on the chosen parameters and what contribution these natural factors make to the overall variation of a biochemical response.

It also appears from the literature that the basis for species selection could be better justified. Obviously, many investigators choose organisms on the rationale of availability or familiarity. If one wants to run any kind of program where biochemical parameters are involved, then species selection needs to take into consideration the commonality, distribution, size and migratory patterns of the species. The use of rare or uncommon fishes may create problems in sample size; therefore, in a field program, a species must be used which is available in adequate numbers. A wide distribution of one or more species perhaps would allow measurements to be made over a greater geographical range, assuming that the populations are all relatively similar in their genetic variation over the same range. Obviously, an adequate size of fish is especially important since one may be drawing blood or collecting certain organs. It would also be important to use a fish species that is relatively non-migratory such as cunner (Bigelow and Schroeder 1953). Throughout the surveyed literature, few papers concerned with field-related effects demonstrated that all four of the above criteria for species selection were fulfilled.

The review of the literature on biochemical parameters also indicated that there are key tissues useful for monitoring work. Among these key tissues are liver, brain and kidney, with serum and gill tissue also being important. Reports of brain tissue studies dominate much of the pesticide-biochemical effect literature due to the large number of studies on the pesticide inhibition of acetylocholinesterase (Johnson 1968). Liver tissue is important because of a variety of effects discussed later, while kidney tissue was described as a good histopathological indicator of pollution. Kidney tissue in fishes is a problem, however, because of hormonal tissue mixed in many areas of the kidney, depending on species. The dispersion of pancreatic tissue in the liver of many fishes is also a problem.

Both serum and gill (especially in view of the interest in gill Na+, K+ ATPase) are also valuable as key tissues. Generally, the surveyed literature favored these five tissues as suitable for biochemical monitoring. However, research into the use of other tissues or systems should be encouraged.

One of the major biochemical responses, evident from the literature, is the effect of pollutants on enzyme systems. The pollutant may have three effects. There may be an inhibition of enzyme activity (Jackim et al. 1970), an induction of enzyme activity (Payne 1976, 1977; Stegeman 1978) or a stimulation of enzyme activity (Jackim et al. 1970). In many cases, the enzyme response may be relatively non-specific (in other words, the enzyme may respond to a variety of pollutants), yet there are excellent examples of specific enzyme response to a specific pollutional type. Examples include the inhibition of acetylcholinesterase (Coppage et al. 1975), the effect of lead on δ-amino levulinic acid dehydratase (Hodson 1976), the induction of benzo(α)pyrene hydroxylase (Payne 1976, 1977). Overall, the usefulness of studying the effects of pollutants on enzyme activity, particularly in the laboratory, is evident. Discussions of extension of these techniques to the field are lacking in the literature except for a few enzyme techniques. Hepatic enzymes appear to be the most useful from a monitoring point of view. However, enzymatic response to a pollutant in a laboratory situation needs to be correlated with pollutant impact in the field. Besides the effects of pollutants on hepatic enzymes, the liver may also respond to pollution through changes in size (Sherwood and Mearns 1977) and coloration (Morgan and Beckerman, in review).

Renal tissue also appears to be a useful indicator for observing histopathological effects of pollution (Stroo and Hook 1977). Relatively little work has been done on renal enzymes in fishes. It has been shown, however, that a variety of pollutants can affect maltase (Stroo and Hook 1977), alkaline phosphatase (Stroo and Hook 1977) and peroxidase (Mukherjee and Bhattacharya 1975).

Another type of biochemical response to pollution is bioaccumulation. This was the most heavily used "biochemical" response in the literature. Tissue residues of either organics or elemental compounds may be used to describe the level and extent of pollution. Examples of residue analysis include the discussions of mercury contamination (Bryan 1976) and polychlorinated biphenyls (Walker 1976).

Bioaccumulation is a fundamental toxicological response dependent on a variety of species-specific and ecosystem parameters (Ariens et al. 1976). It is most useful in describing the pollutant and the level of contamination.

A promising bioaccumulation tool may be the analysis of bile in fishes. Generally, lipid soluble compounds may accumulate in the liver, gonads or other tissues in fishes where high lipid concentrations occur. The problem in aquatic environments is assessing the ecological impact of compounds with low lipid solubilities. Many of these compounds are as dangerous ecologically as the lipid soluble compounds, but may not be persistent. Statham et al. (1976) have found that biliary-hepatic function tends to concentrate compounds of low lipid solubility in the bile. Collection of bile in the field may allow characterization of the presence of low-level non-lipid soluble contaminants in aquatic ecosystems.

Histopathology also appears to be useful for describing pollutional effects either at the level of light or electron microscopy. However, the majority of pollutional effects on fish tissues are generally non-specific. The coupling of scanning electron microscopy with X-ray probe analysis may be useful in the future for analyzing tissue damage due to elemental accumulation.

Other promising techniques for the analysis of pollutional effects on biochemical responses include changes in corticosteroid concentrations (Donaldson and Dye 1975), although problems may occur (Schreck and Lorz 1978). The effect of pollutants on endocrine and exocrine systems is understudied in fishes, yet pollutional effects on endocrine systems may have far-reaching consequences.

The use of biochemical responses in monitoring pollution in the field still has many problems. Sensitivity and non-specificity appear to be two of the major problems in the future use of biochemical monitoring. Another problem is the singular approach, utilizing either one test or one tissue, whereas the pollutant may be affecting a variety of physiological-biochemical systems and the discrimination of a specific pollutant may require analyses of its effects on different biochemical systems.

Another problem in the use of any biochemical test is the cost/time problem. This problem occurs because not only is the collection of samples required, but also the handling, storage, laboratory preparation and analysis, and finally interpretation of perhaps a large set of samples from many species and many tissues. Based on my own experience, the time required to process a field sample versus a comparative sample for biochemical effects is approximately in the ratio of 1:3 or 1:4. In essence, the work in the laboratory far exceeds the work spent in the field collection (assuming data analyses and interpretation time are equivalent for the two studies). Finally, another problem may be in the lack of trained ecophysiopathologists.

In the beginning, I asked the simple question, "Are biochemical parameters useful in describing or isolating pollution stresses in fishes?" After a review of the available literature, the answer to me appears to be a simple "maybe".

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ABSTRACT

The adenylate energy charge (AEC = [ATP + 1/2 ADP]/[ATP + ADP + AMP]) is a measure of the metabolic energy available to an organism at the time of sampling. Values, which range between 0 and 1, have been correlated with physiological condition and growth state: namely high values (0.8 - 0.9) in healthy organisms under optimal or normal conditions, and reduced values in organisms in non-optimal or stressed environmental conditions. These data indicate that AEC may be useful as an indicator of stress. The AEC of two species was, therefore, measured under a variety of laboratory and field conditions to assess the potential of AEC as a stress indicator.

In 2 estuarine, molluscan species, (Pyrazus ebeninus, gastropoda, and Trichomya hirsuta, bivalvia), AEC values were consistently between 0.8 and 0.9 in control animals. Values were significantly less (between 0.55 and 0.65) than controls under conditions of reduced salinity, increased temperature, exposure to hydrocarbons and exposure to power station outfall.

These results demonstrated several advantages that AEC measurements have over more conventional bioassay methods. These include rapidity of response, low variability and relative ease of measurement. These advantages, as well as several methodology-related problems, are discussed in relation to the use of AEC in toxicity monitoring programmes.

Key words: Adenylate energy charge, AEC, stress, stress indicator, molluscs, environmental monitoring.

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INTRODUCTION

Although a number of techniques have been developed in an attempt to evaluate the "well-being" of organisms under a variety of sub-lethal environmental conditions (e.g., Sprague, 1971; Swartz, 1972; Waldichuk, 1973; Vernberg and Vernberg, 1974; McCleay and Howard, 1977; McIntyre et al., 1978), their application has had limited success. Some are limited because the high levels of variability between individual organisms require many replicates before treatment effects can be detected statistically. Some are limited to certain species or phyletic groups. Some are not consistent in response and thus lack predictive power. Others require lengthy periods of exposure before responses are detectable.

This paper describes a biochemical measure known as the adenylate energy charge (AEC). This measure offers a number of significant advantages (described below) over methods which are currently used to evaluate an organism's response to environmental perturbation. Results obtained with two estuarine invertebrate species are presented to illustrate how it might be applied in toxicity evaluation. These results are summarized from Ivanovici (1977, 1979a,b and c). Several limitations which have become apparent from this and other work are also discussed.

THE ADENYLATE ENERGY CHARGE - BACKGROUND

The AEC is defined as the amount of metabolic energy available to an organism from the adenylate pool (Atkinson, 1968, 1977). It is calculated from measured concentrations of the 3 adenine nucleotides, adenosine triphosphate (ATP), -diphosphate (ADP), and -monophosphate (AMP), which are integral to the energy metabolism of all organisms. The AEC is calculated by the formula:

$$\frac{(\mathrm{ATP} + \tfrac{1}{2}\,\mathrm{ADP})}{(\mathrm{ATP} + \mathrm{ADP} + \mathrm{AMP})}$$

By definition, the AEC has a specific range of values from 0 to 1. From data available in the literature, the AEC appeared to offer 4 advantages over other methods:

(a) The ubiquitous distribution of the adenine nucleotides meant that it could be measured in any organism - from bacteria to plants, invertebrates and mammals (e.g., Chapman et al., 1971).

(b) Variation between individuals was less than reported for other measures such as, for example, the individual nucleotides themselves (e.g., Ballard, 1971; Ching et al., 1975) or serum glucose and protein levels (Telford, 1968; Stewart & Li, 1969).

(c) Specific values of AEC obtained by a number of investigators correlated with physiological state or "well-being" in a variety of organisms (as summarized in Table 1).

Adenylate Energy Charge	Environmental Condition	Organisms Characterized by:
0.80 - 0.90	Non-limiting (no stress)	-high growth rate -reproduction -viability
∿0.50 - 0.75	Limiting (partial stress)	-slow or zero growth rate -no reproduction -viability maintained
∿<0.50	Severely limiting (severe stress)	-no growth -no reproduction -viability lost, even after transfer to non-stress conditions

Table 1. Values of adenylate energy charge, their association with specific environmental conditions, and the characteristics of organisms under these environmental conditions.

High values (0.8 to 0.9) were typical for organisms under optimal conditions (e.g., *Escherichia coli*, Chapman *et al*., 1971; rats, Ridge, 1972; soybean nodules, Ching *et al*., 1975; bivalve mollusc, *Mytilus edulis*, Wijsman, 1976). Where examined, organisms with high AECs had high rates of growth and the ability to reproduce. For example, Simmonds and Dumbroff (1974) showed that a high AEC was a prerequisite for maximal axis elongation in seeds of *Acer saccharum* treated with growth hormones. AECs between 0.5 to 0.7 have been found in many organisms exposed to environmental perturbations which were known to be limiting or partially stressful. Lower AECs have been reported in microorganisms (e.g., *E. coli* in nutrient-limited media, Chapman *et al*., 1971), plants (e.g., anoxia effects on soybean nodules, Ching *et al*., 1975; dessication effects on the moss *Tortula ruralis*, Bewley and Gwozdz, 1975), invertebrates (e.g., effect of reduction in salinity on the parasite *Monieza expansa*, Behm and Bryant, 1975, and the estuarine mollusc *Pyrazus ebeninus*, Ivanovici, 1974; effect of anoxia on the mollusc, *Mytilus edulis*, Wijsman, 1976) and vertebrates (anoxic effect on the goldfish *Carassius auratus*, Thillart *et al*., 1976, and rats, Ridge, 1972). The lower AECs correlated with slower growth rate (e.g., Chapman *et al*., 1971; Wiebe and Bancroft, 1975). Studies by Chapman *et al*. (1971), Ridge (1972), and Montague and Dawes (1974) also demonstrated that an organism's viability or its ability to recover, after removal from the partial stress condition was related to how low the AEC decreased. If values fell below approximately 0.5, viability was lost, and the organisms did not recover on return to optimal conditions. If values remained above this critical threshold, growth and reproduction was resumed.

(d) Response times for AEC were fast, ranging from minutes in microorganisms (Chapman *et al*., 1971; Wiebe and Bancroft, 1975) up to 24 h in some multicellular organisms (Ridge, 1972; Wijsman, 1976). Where response times exceeded 24 h, the AEC response occurred well before changes in other physiological measures (Giesy *et al.*, 1978).

METHODOLOGY

Experiments were done with adults of two estuarine molluscan species: the gastropod, *Pyrazus ebeninus*, and the bivalve, *Trichomya hirsuta*. Both species are abundant in estuaries of the east coast of Australia. The distribution of *P. ebeninus* tends to be in the more saline parts of the estuary, while *T. hirsuta* is found in more brackish areas. Descriptions of experiments are given in detail by Ivanovici (1979a & b) and summarized in the legends of tables which follow.

The procedures used to extract and analyze ATP, ADP and AMP are summarized in Fig. 1, and are detailed elsewhere (Ivanovici, 1979*d*).

RESULTS

PYRAZUS EBENINUS

Variation Over Tidal Cycle

Measurement of AEC in *P. ebeninus* collected in the field at high and low tides were consistently between 0.8 and 0.9 (Table 2), with significantly lower values at low tide and after a period of heavy rains (which resulted in abnormally low salinities in the estuary).

TIDE	AEC ± 95% C.L.
High	0.87(4x5) ± 0.03
Low	0.79(4x5) ± 0.03
Flood	0.63(12) ± 0.06

Table 2. AECs of *P. ebeninus* collected in the field at high and low tides, and after a period of prolonged rain in the estuary (Flood). Values are means of 20 (samples of 5 animals collected at 4 different times) or 12 animals ± 95% confidence limit (C.L.) calculated from analysis of variance (from Ivanovici, 1979*b*).

Laboratory Studies

Under laboratory conditions, the AEC did not change in *P. ebeninus* kept at salinities between 25 and 40‰ (Fig. 2). The molluscs were active at these salinities which were similar to those measured at high tide in the field when the molluscs were also observed to be quite active. Significantly reduced ($p<0.005$) AECs were measured in animals kept in salinities of less than 20‰ within 24 h. An additional significant reduction occurred after 7 or more days, but the change was only half that of the initial change. Irrespective of the

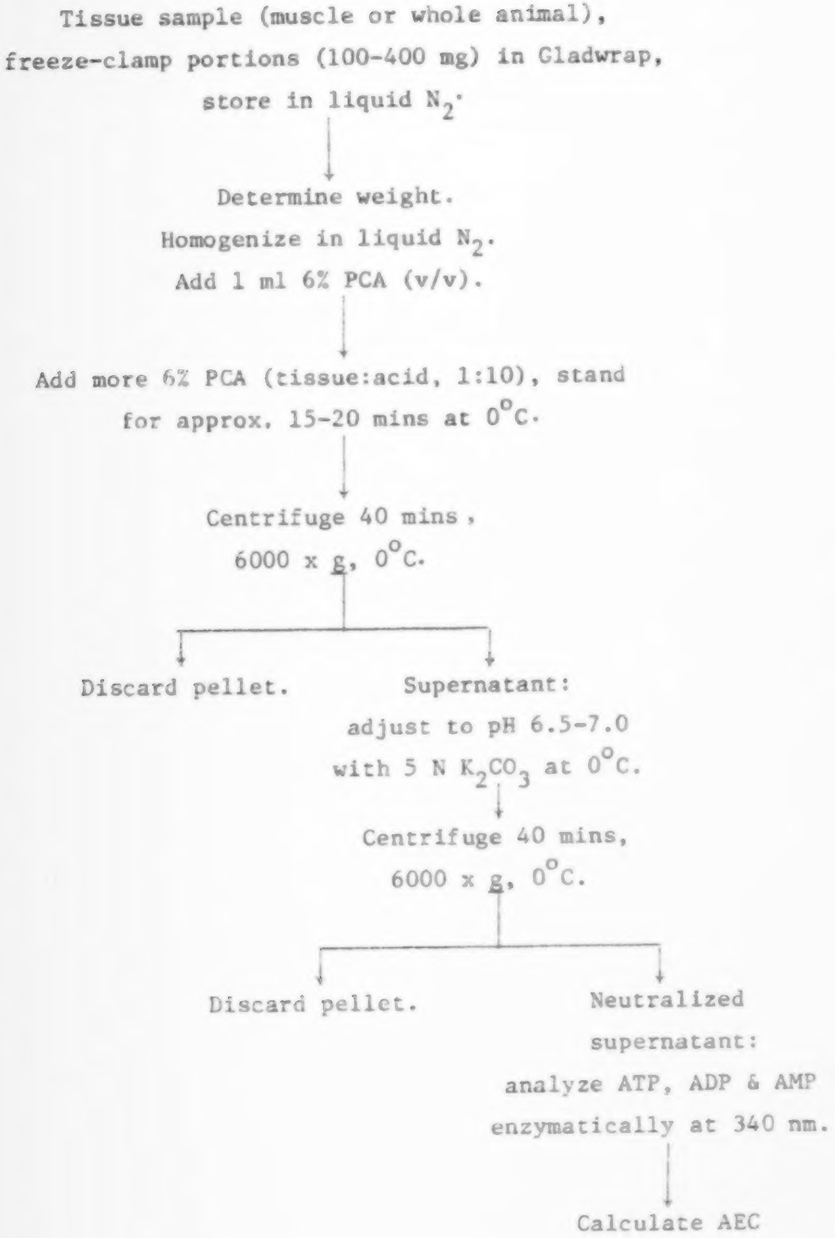
Tissue sample (muscle or whole animal),
freeze-clamp portions (100-400 mg) in Gladwrap,
store in liquid N_2.
Determine weight.
Homogenize in liquid N_2.
Add 1 ml 6% PCA (v/v).
Add more 6% PCA (tissue:acid, 1:10), stand
for approx. 15-20 mins at 0°C.
Centrifuge 40 mins,
6000 x g, 0°C.
Discard pellet.
Supernatant:
adjust to pH 6.5-7.0
with 5 N K_2CO_3 at 0°C.
Centrifuge 40 mins,
6000 x g, 0°C.
Discard pellet.
Neutralized
supernatant:
analyze ATP, ADP & AMP
enzymatically at 340 nm.
Calculate AEC

Fig. 1. Summary of procedure for the determination of adenine nucleotides and adenylate energy charge in molluscan tissues. PCA, perchloric acid; Gladwrap is a plastic film used as food wrapping; it was found very suitable and preferred for this procedure (from Ivanovici, 1977 & 1979d).

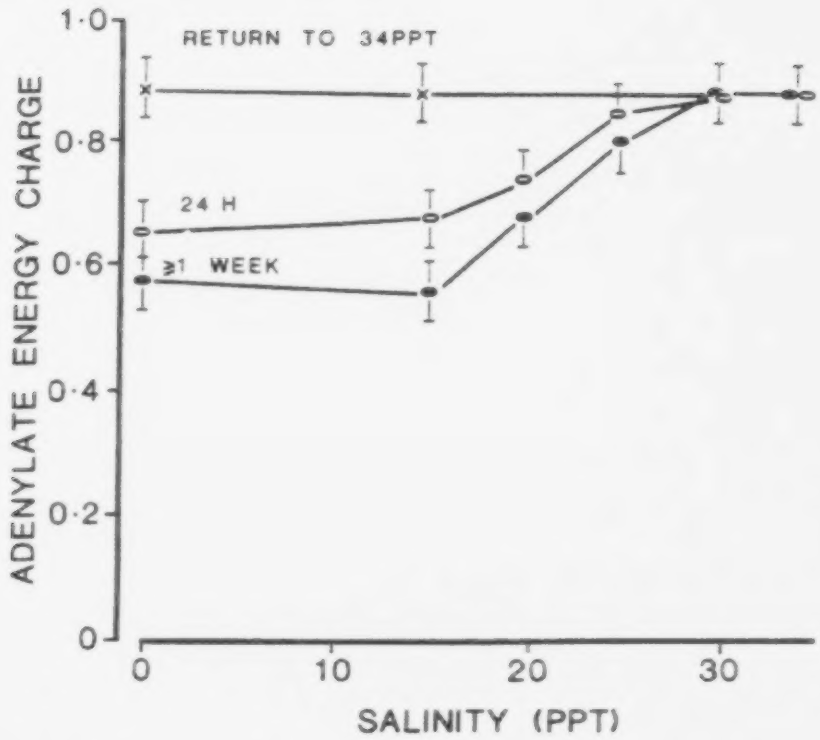

Fig. 2. The adenylate energy charge of *Pyrazus ebeninus* at various salinities at 20°C. The response was significant only after exposure for 24 h (○), with further significant but smaller reduction if exposure period was extended to 7 days or more (●). Molluscs that were returned to 34°/oo from 0 and 17°/oo regained high charge values (x), irrespective of the salinity or exposure time. Each point is the mean of at least 3 animals. Bars are the 95% C.L. calculated from analysis of variance (from Ivanovici, 1979*a*).

exposure period (up to 3 weeks) and salinity, AECs returned to control levels when molluscs were returned to 34^{o}/oo.

An increase in temperature (from 20° to 29°) did not alter the AEC response to salinity (Fig. 3), but values were approximately 20% less at all salinities. The AECs of molluscs at the two low salinities (0 and 17^{o}/oo) decreased to the critical region of 0.5 to 0.55 within 24 h. Animals returned from the low salinities to control conditions did not recover. While 29°C is not an uncommon air temperature during summer in areas where *P. ebeninus* are found, the molluscs experience this temperature intermittently only (i.e., at low tide, during the day). This was reflected by the low AECs.

Field Experiments

The ultimate value of a monitoring method is best tested by applying it to field situations, where environmental factors are not as controlled as under laboratory conditions. Two experiments were done to determine if reductions in AEC could be correlated with man-induced deterioration of environment. Molluscs were, therefore, transferred to sites in the Parramatta River (Sydney) which were contaminated with hydrocarbons (Furzer, 1975). The most contaminated site was at Duck River, the least, Morrison's Bay and the control sites (the collection estuary, Moona Moona Creek, and a nearby one, Currambene Creek, in Jervis Bay, New South Wales). Details of these experiments are fully documented elsewhere (Ivanovici, 1979*b*). The first trial (Table 3) showed that the AECs of all animals transferred to the Parramatta River were significantly less ($p<0.05$) than for animals at the control site. Furthermore, the greatest reduction was found in animals kept at the most heavily contaminated site (Duck River). This reduction occurred within 24 h of transfer, and was not associated with either water temperature or salinities.

Site	Adenylate Energy Charge ± 95% C.L.	
Moona Moona Creek Jervis Bay (control)	0.81(5x6)	±0.06
Morrison's Bay Parramatta River	0.75(4x6)	
Ermington Bay Parramatta River	0.73(4x6)	
Duck River Parramatta River	0.64(4x6)	

Table 3. Adenylate energy charges of *P. ebeninus* transferred to sites contaminated with hydrocarbons. Samples of 6 animals were taken at 0 (control only), 24 h, 1, 2 and 3 weeks. The least contaminated site was at Jervis Bay (control), the most contaminated site at Duck River. Numbers in brackets represent the number of time samples by the number of animals sampled at each time. Times have been pooled to give site means (from Ivanovici, 1979*b*).

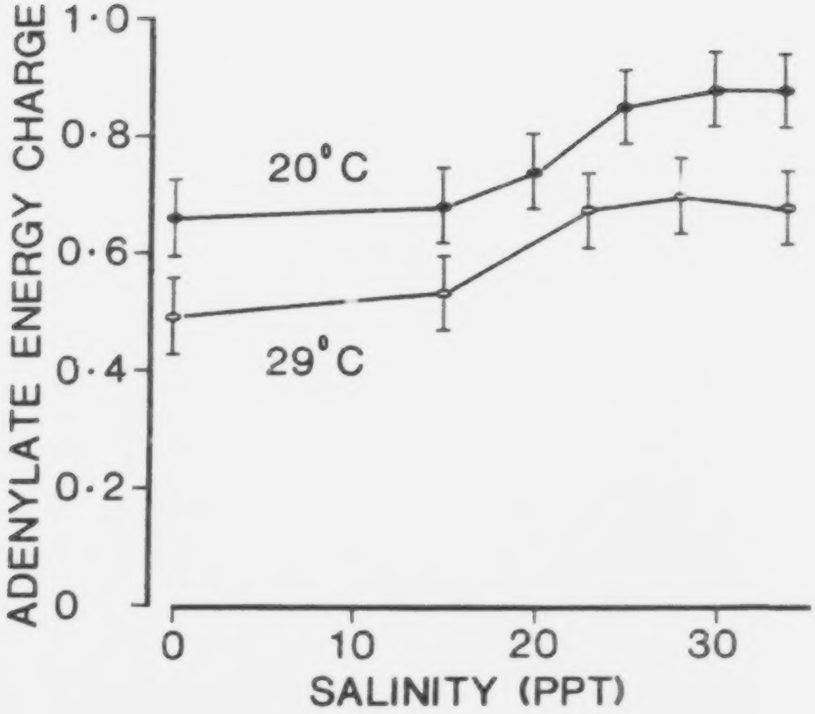

Fig. 3. The adenylate energy charge of Pyrazus ebeninus at various salinities at 20°C and 29°C. The response was significant within 24 h and did not change significantly over the period of the experiment (4 days). Each point at 20°C is the mean of 6 animals, and at 29°C is the mean of 5 animals. Bars are the 95% C.L. calculated from analysis of variance (from Ivanovici, 1979a).

A second transfer experiment (Table 4) again demonstrated significantly reduced AECs in animals moved to the Parramatta River. AECs of molluscs that remained in the "home" estuary (Moona Moona Creek) or were moved to an adjacent "clean" estuary (Currambene Creek) did not change over the experimental period. Molluscs which were returned from the Parramatta River sites to the home estuary showed significant increases of AECs back to control levels. AECs of the group which had been moved to Currambene Creek did not alter during the experimental period. Of 30 molluscs that were left at each site at the completion of the experiment, no live ones were found at any of the Parramatta River sites after nine months.

	Adenylate Energy Charge		
	Time (Weeks)		
Site	0	2[a]	4
Moona Moona Creek (control)	0.76	0.82	0.81
Currambene Creek (Control)		0.80	0.77
Morrison's Bay (Parramatta River)		0.62	0.79
Duck River (Parramatta River)		0.69	0.80
	95% C.L. ± 0.02		

Table 4. Adenylate energy charge of P. ebeninus transferred to nonpolluted and polluted sites. After a 2 week exposure, a, transferred molluscs were returned to Moona Moona Creek and sampled 2 weeks later (week 4). Values are means of 6 animals. The 95% C.L. was calculated from the analysis of variance (from Ivanovici, 1979b).

TRICHOMYA HIRSUTA

The value of a monitoring method is enhanced if it can be applied to more than one species with similar results. AEC measurements were, therefore, made on another common estuarine species, the bivalve, Trichomya hirsuta. In this case, samples were collected from the inlet and outlet sides of water-cooled electric power station (Vales Point, Lake MacQuarie, New South Wales). While animals were abundant on the inlet side, they were difficult to locate on the outlet side. The AECs of bivalves from the outlet side, where water temperature was ∿8°C higher than the water on the inlet side, were significantly less than in animals from the inlet (Table 5).

	VALES POINT POWER STATION	
	Inlet	Outlet
Adenylate Energy Charge	0.78	0.68
95% C.L.	±0.04	
Temperature (°C)	21.2	28.9
Salinity (°/oo)	30.7	31.0

Table 5. Adenylate energy charges of *Trichomya hirsuta* from inlet and outlet sides of a power station. Values are means of 6 animals. The 95% C.L. was obtained from analysis of variance (data from Ivanovici, 1979*c*).

DISCUSSION

The measurements of AEC obtained in *P. ebeninus* over the tidal cycle (Table 2) indicated that, while the AEC fluctuated with naturally occurring changes in the estuary, values remained between 0.8 and 0.9, i.e., within the range predicted by Atkinson (1968,1977), except when the perturbation was excessive (as was the case with prolonged low salinity after heavy rains).

In the laboratory, AECs of *P. ebeninus* decreased significantly with reductions in salinity below 20°/oo (Fig. 2) or an increase of temperature by 9°C (Fig. 3). Apart from Behm and Bryanc's (1975) study of the tapeworm *M. expansa*, where AEC was found to decrease under reduced osmotic conditions, and a preliminary study of *P. ebeninus* (Ivanovici, 1974), the response of AEC to important environmental factors in estuaries, such as salinity and temperature, have not previously been described. Given that the AEC reflects less available metabolic energy at the low salinities, the lower AECs offer some insight into the distribution of this species within the estuarine environment. *P. ebeninus* is found only near the mouths of estuaries or where the salinity is >25°/oo for at least part of the tidal cycle. Where salinities are lower, the metabolic cost is too great for survival. The AEC response at 29°C supports Bayne's hypothesis (1973) that increases in temperature of as little as 8°C above acclimation temperature could be sufficient to place molluscs "out of energy balance" and under stress. The lower AECs found in the population of *T. hirsuta* on the outlet site of the power station also support this.

These results confirmed that AEC measurements did not have some of the problems shown by other biochemical methods, as discussed in "the Adenylate Energy Charge - Background" section.

1. Lower AECs were consistent with environmental perturbation, whether in the laboratory or in the field.
2. Variability between individuals was sufficiently low to require sample sizes of as few as 4 to 6 animals to detect significant differences between treatments. Furthermore, variability levels (as indicated by 95% confidence limits) were not significantly greater in the field than under laboratory conditions.

3. The response was fast, generally occurring within 24 h - both under field and laboratory conditions.

4. The response was demonstrated in two unrelated species.

Although these data indicate that AEC measurements have potential in the monitoring of pollution effects, their use must be approached cautiously. The reasons for this relate to:

(a) methodology;

(b) exceptions in the response pattern of AEC to non-optimal conditions; and

(c) lack of data relating the effect of reduced AECs to fecundity and viability of offspring.

The highly labile nature of ATP in biological samples necessitates careful evaluation of each stage of processing. The effects of time delays in handling and dissection on ATP and AEC levels of the organism before inactivation of enzymes (by freeze-clamping at -180°C) must be determined, as must be the efficiency of extraction of the adenine nucleotides from the sample, and possible inhibitory or stimulatory effects by the extractant on the three assays. The first two must be established with initial experiments and need to be done for every test species. In addition, great care is needed during sample extraction and analysis to prevent breakdown of ATP. Correct temperatures for each step must be maintained, as well as pH. This means that the method, as it is at present, is labour intensive and time consuming. For example, 28 individual organisms can be processed (i.e., extracted and nucleotides analyzed and calculated) in two days by two people - once the effects of handling and the extraction efficiencies are known (personal experience). Ching (personal communication) reports that 18 replicate samples of plant material can be completed in two days.

The method for sample inactivation must be carefully selected because enzyme activity can lead to changes in the adenine nucleotides, which in turn can result in erroneous in vivo estimates. In the present example, freeze-clamps were used (aluminum blocks cooled to -180°C). These are recommended by many workers (e.g., Bergmeyer, 1974), but may not be the most appropriate method for some species. For example, Wadley et al. (1979) found that estimates were more consistent when 2 bivalve species were frozen whole rather than dissected and clamped. Extraction efficiency may vary with the extracting medium. In the present example, cold perchloric acid (PCA) was found to be most suitable for the tested species. Other workers have, however, found that other media were better for their studies. For example, Wijsman (1976) found trichloroacetic acid was more suitable than PCA, but Wadley et al. (1979) found that PCA was superior to trichloroacetic and sulphuric acids, and boiling bicarbonate buffer.

There are several examples in the literature which indicate that the response of AEC to stress is not entirely consistent with that outlined in "The Adenylate Energy Charge - Background" section. Values of AEC less than 0.8 have been measured in actively growing cells (e.g., Eigener,1975), in animals under normal conditions (Zs.-Nagy and Erminy, 1972) and in different organs of animals (Wijsman, 1976). Some organisms remain viable despite AECs below 0.5

(Ball and Atkinson, 1975). High AECs have been measured in moribund organisms which were under known lethal conditions (Chapman and Atkinson, 1973; Ivanovici, unpublished data). While some of these examples can be attributed to inadequately controlled or inappropriate methodologies (Knowles, 1977), or to an organism's spore-forming capability (Knowles, 1977), other examples (such as the maintenance of high AECs under severe stress) suggest that this method may simply be inappropriate for some species. Species, then, need to be selected for their ability to demonstrate a reduced AEC, as well as their commercial and/or biological importance.

The final limitation of AEC which will be considered in this paper concerns the predictive power of AEC measurements. A few studies of microorganisms have shown that reductions in AEC affect growth characteristics and viability (Chapman _et al_., 1971; Montague and Dawes, 1974). Apart from Ridge's (1972) study on anoxic effects on rats, however, little is known about multicellular organisms. It would be useful to know, for example, what affect short-term and long-term decreases of AEC have on the reproductive capacity of stressed organisms and the viability of their offspring. Studies in this area still need to be done, and would establish the predictive power of AEC measurements.

In conclusion, while a number of problems are associated with AEC measurements and their interpretation, useful information can be obtained as long as these problems are recognized and appropriate controls incorporated into experiments. Several examples were given here to illustrate the AEC response, and how it helped to understand better the biology of the test species. Further work, therefore, is encouraged, but with caution.

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ABSTRACT ONLY

The mixed function oxidases (MFOs) are a group of enzymes usually reported and studied in the endoplasmic reticulum of vertebrate liver, though they are not necessarily restricted to that tissue. They catalyse the degradation of both endogenous and exogenous compounds, generally by oxidative processes, and generally in the direction of non-polar compounds to polar compounds: thus they contribute to the clearance of non-polar compounds as polar metabolites from organisms. They are inducible: their activity can be increased by exposing the organism to various potentially toxic compounds such as organochlorine pesticides, petroleum hydrocarbons, drugs, etc. They are generally regarded as being part of a defence system available to the organism when it is "stressed" by such compounds. In principle, the activity of MFOs might be used as a general index of the stress caused by various chemicals - in short, a non-specific bioassay of sub-lethal stress.

In this paper, I discuss the effects on trout (Salvelinus fontinalis) hepatic MFOs of feeding various inducing compounds, which are known or expected environmental contaminants. DDT and DDE did not induce the MFO system, but PCBs, PCTs and aromatic hydrocarbons (typified by 3-methylcholanthrene) induced various components of MFO systems, most notably the activity of the enzyme ethoxycoumarin O-de-ethylase. The differences between compounds probably reflect different mechanisms of induction: compounds such as DDT and DDE in mammals induce a MFO system in which cytochrome P-450 is the terminal oxidase, but have no effect on the trout system. However, compounds which in mammals induce a system involving cytochrome P-448 will induce the trout MFO system. Thus it appears that the trout MFO system may have some uses as a bioassay tool for the latter groups of compounds.

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Malley[*], D.F., W. Dentry[**], and S.L. Leonhard[*]. 1979. Calcium uptake by postmoult Daphnia magna: A potential sublethal toxicity test. Proc. Fifth Annual Aquatic Toxicity Workshop, Hamilton, Ontario. Nov. 7-9, 1978. Fish. Mar. Serv. Tech. Rep. 862, pp. 257-265.

ABSTRACT

A technique is described for the measurement of calcium uptake by individual postmoult Daphnia magna. This uptake may serve for Daphnia magna, as it does for certain crayfish, as a basis for testing the toxicity of low pH and low pH-heavy metal combination. Adult D. magna in the postmoult stage were obtained removing from the mass culture females bearing mature ephippia and observing them until they moulted within a few hours to release the ephippium. In culture these females continue to survive and produce parthenogenetic broods. The medium, 200 µL in volume, containing a newly-moulted female and spiked with 0.2 to 0.3 µCi $^{45}CaCl$ is sampled for changes in the ^{45}Ca content with time for 3 hours. The counts per minute of ^{45}Ca, transformed to natural logarithms are expressed as a function of time and fitted with a straight line. The slope of this line, b_{normal}, is compared with the slope of this relationship following addition of the toxicant(s), b_{tox}, as follows:

$$\text{per cent inhibition} = \left(1 - \frac{b_{tox}}{b_{normal}}\right) \times 100\%$$

Key words: Daphnia magna, toxicity test, postmoult calcium uptake, ephippium.

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INTRODUCTION

Daphnids, such as *Daphnia magna* (Crustacea: Branchipoda: Diplostraca: Cladocera), are widely used in freshwater toxicology and water quality testing for reasons outlined by Adema (1978). *Daphnia magna* is easily cultured, available throughout the year, can practicably be tested through several generations and is the largest and easiest daphnid to handle. Daphnids are a very important link in freshwater food chains worldwide (Crosby and Tucker 1966).

Most studies on daphnids measure bioaccumulation of substances (Crosby and Tucker 1971; Johnson et al. 1971; Reinert 1972; Sanders and Chandler 1972) or effects of toxicants on survival or reproduction (Davis and Ozburn 1969; Winner and Farrel 1976; Adema 1978; Canton and Adema 1978). Less frequently effects on growth are observed (Beisinger and Christensen 1972). Survival, reproduction and growth are the outcomes of highly complex sets of processes. If they are inhibited, it may not be clear at what point or by what mechanism the toxicant has acted. It is desirable, in addition, to develop toxicity tests based on more discrete physiological functions. Activities of certain enzymes have been used as the basis of toxicity tests (Beisinger and Christensen 1972; Dortland 1978) but the biological significance of the toxicant effect is often not clear.

Uptake of calcium by postmoult *Daphnia magna* is suggested here as a potential sublethal toxicity test. Calcium uptake during the postmoult period has been used to test effects of low pH, cadmium and selenite on the crayfish *Orconectes virilis* (Malley a, in preparation).

The uptake of calcium from the environment that occurs during the postmoult period in crustaceans is large compared with the movements of other physiological ions because of the large amount required for calcification of the new exoskeleton. Thus, Ca^{++} movements are relatively easier to measure with radioactive tracers than other ions. Toxicants which inhibit postmoult Ca^{++} uptake can be expected to interfere with calcification and may also affect ionic regulation and acid-base balance.

MATERIAL AND METHODS

The methods used for the mass culture of *Daphnia magna* are described by Leonhard and Lawrence (a, in preparation). The culture medium was prepared to contain the following in mMoles L^{-1}: K^+, 0.78; Na^+, 0.73; Ca^{++}, 0.70; Mg^{++}, 0.16; Cl^-, 2.07; $SO_4^=$, 0.16. pH was adjusted to 6.65. The production of ephippia by the *Daphnia* was induced in the mass culture by allowing the medium to become more concentrated and by ceasing to feed. *Daphnia magna* which would moult within a few hours were recognized by the mature appearance of the ephippium, dark in colour, large and slightly separated from the female. More than the number of individuals required were isolated from the mass culture tank and observed at five-minute intervals until sufficient numbers had moulted. Usually within one to five minutes of the moult each *Daphnia* was transferred with 200 µL of its medium to a 1/8 dram glass vial. Vials without *Daphnia* containing 200 µL of the medium served as controls. Also tested were *Daphnia* which had moulted 24 to 48 hours previously and a premoult *Daphnia*. To each vial was added 5 µL of ^{45}CaCl solution containing 0.2 to 0.3 µCi. ^{45}Ca in the test medium was measured as counts per minute (CPM) over time by removing 5 µL aliquots at 5 to 20 minute intervals over a total time of up to three hours. The experiments were carried out at 23±1°C. ^{45}Ca was measured by scintillation counting in 15 mL of Amersham PCS fluor. Since half life of ^{45}Ca is 164 days and all aliquots from a single *Daphnia* were counted within two hours of each other, no correction was made for radioactive decay. Quenching was assumed constant for all samples. ^{45}Ca was counted with 80% efficiency.

The initial concentration of Ca^{++} in the 200 µL test media varied from 0.34 to 0.85 mMoles L^{-1}. Some *Daphnia* moulted and were tested in unmodified medium from the culture tank. Due to evaporation, the Ca^{++} concentration in this tank reached as high as 0.85 mMoles L^{-1}. Other *Daphnia*, after removal from the culture tank, were gradually exposed, over a several hour period before they moulted, to lowered Ca^{++} levels by mixing the culture medium with a low Ca^{++} medium. The latter had the ionic composition indicated above except for Ca^{++}, 0.1, and Cl^{-}, 0.87 mMoles L^{-1}.

Dry weights of *Daphnia* were determined on individuals from the same mass culture tank as those for the ^{45}Ca uptake studies. Within minutes of their moult to release the ephippium, they were exposed to air at room temperature for 36 hours, then dried at 97±2°C for 50 minutes. Individuals were weighed to the nearest 0.1 µg.

Natural logarithms of CPM of ^{45}Ca corrected for background counts were plotted as a function of time in hours for each *Daphnia* and fitted with a straight line: ln CPM = a + b(time). The statistical significance of each slope ($b \neq 0$) was tested by means of Student's t-test (Steel and Torrie 1960). Significance was accepted at the 0.05 level. In those cases where $b \neq 0$, the initial rate at which those *Daphnia* were taking up Ca^{++}, termed the initial influx, was calculated from:

$$-M = b(C)$$

where M is the initial influx in nanomoles hr^{-1} individual^{-1}, b is the slope of the line mentioned above and C is the initial amount of Ca^{++} in the vial. This equation used by Malley (b, in preparation) was derived from the relationship given by Shaw (1959):

$$\frac{dy}{dt} = - \left(\frac{M}{C}\right)y$$

where dy/dt is the rate of decrease of radioactivity and y is the relative radioactivity per unit volume of the solution.

Analysis of the Ca^{++} concentration in the media was performed by the Chemistry Unit of the Freshwater Institute by atomic absorption spectrophotometry using the methods of Stainton et al. (1977).

RESULTS

Calcium uptake was measured individually on 21 postmoult *Daphnia magna* beginning from 1 to 30 minutes after the moult for periods ranging from 1.5 to 2.9 hours. In fourteen (67%) of these cases, CPM of ^{45}Ca declined in the medium as shown for a typical individual in Fig. 1. Calcium was calculated to be

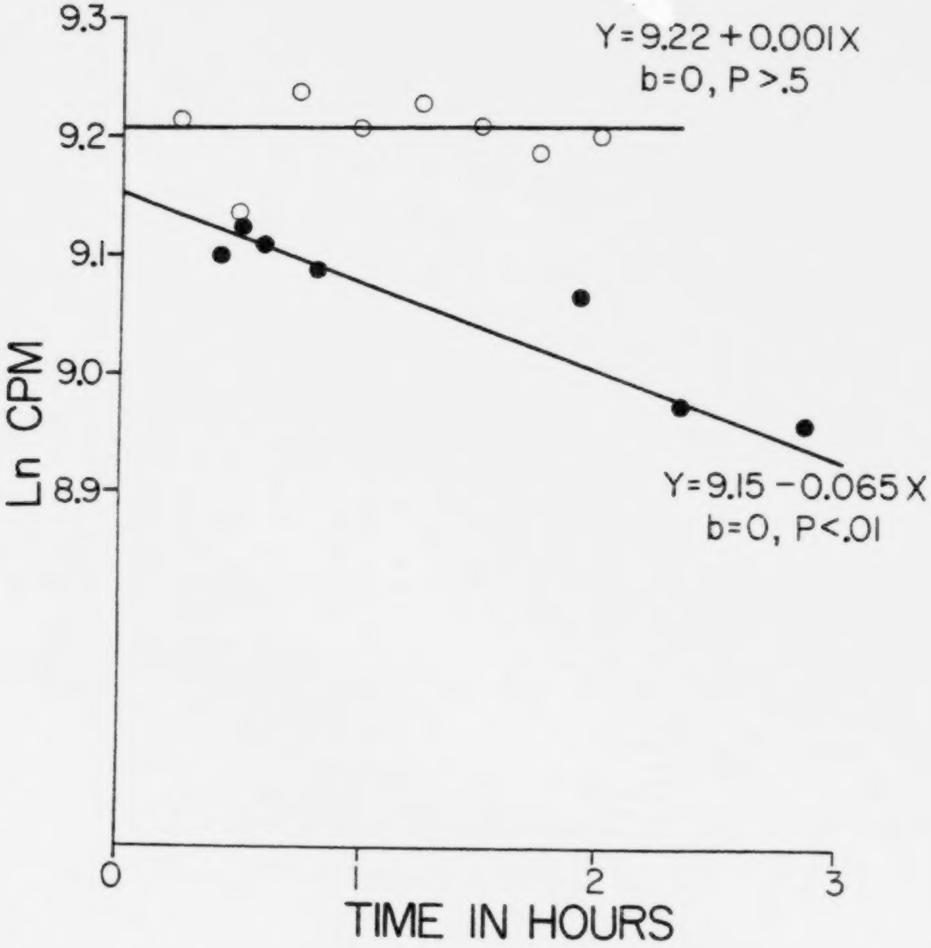

Fig. 1. The relationship between the natural logarithm of the CPM of ^{45}Ca in a 5 µL aliquot of the medium and the time after the addition of the radioactive label. ● represent values in the presence of a post-moult *D. magna*. O represent values for a control vial with no *D. magna*.

moving into these 14 *Daphnia* at initial influx rates ranging from 5.2 to 13.1 nanomoles hr^{-1} $individual^{-1}$. Mean dry weight ± S.D. of 18 postmoult *Daphnia* was 0.0647±0.0105 mg. Expressing the initial influx on the basis of the mean dry weight gives values of 80.4 to 202.5 μMoles Ca^{++} g^{-1} hr^{-1}. Four (19%) *Daphnia* became entrapped in the surface film and showed no uptake of ^{45}Ca from the medium. Three individuals (14%) produced variable CPM of ^{45}Ca which showed no statistically significant change with time. Of 11 control vials without *Daphnia*, nine showed no change in CPM with time (Fig. 1), one showed a statistically significant increase and one a decrease.

Also tested were two *Daphnia* which had moulted from 24 to 48 hours previously and a premoult animal which did not moult during the period of measurement. None of these took up calcium.

The initial calcium concentration in the test media, which ranged from 0.34 to 0.85 $mMolesL^{-1}$, influenced the intial influx of Ca^{++} into *Daphnia* as shown in Fig. 2. for the 14 *Daphnia* mentioned above ($r = 0.87$, $P < 0.01$).

DISCUSSION

This paper describes a technique for measurement of calcium uptake of individual *Daphnia* which does not require that the animal be sacrificed. The effect of a toxicant on calcium uptake can be measured by comparing rate of calcium uptake before and after the toxicant is applied. The animal serves as its own control and variation between individuals in the rate of postmoult Ca^{++} uptake need not be considered. The calculation for expressing the effect of a toxicant on the Ca^{++} uptake has been described by Malley (b, in preparation). The slope of the line relating ln CPM of ^{45}Ca to time is first determined in the absence of the toxicant, b_{normal}, and then remeasured after the toxicant has been added, b_{tox}. The toxic effect is expressed as per cent inhibition:

$$\text{per cent inhibition} = \left(1 - \frac{b_{tox}}{b_{normal}}\right) \times 100\%$$

Several problems were encountered in developing this technique. Evaporation of the medium during measurement of Ca^{++} uptake can be reduced by use of tall vials so that the surface area open to the air is minimal and by capping the vials between samplings. Entrapment of *Daphnia* in the surface film occurred in 19% of our cases. Care must be taken in transferring the *Daphnia* to the test vial to avoid air bubbles under the carapace. A non-ionic surfactant such as Tween 80 may be used, but its effect on Ca^{++} should first be tested. Tween 80 between 0.001 and 0.0001% (w/v) is the least toxic to *Daphnia* of synthetic surfactants studied by D. F. Gerson and S. Krause (pers. comm.)

Initially in the development of this method early postmoult *D. magna* were obtained by screening out newly-released juveniles at the first instar stage from the mass culture at one-hour intervals. Eight hours later each batch of *Daphnia* moulted nearly synchronously and groups at the second instar stage were available for measurement of Ca^{++} uptake. To obtain sufficient live biomass

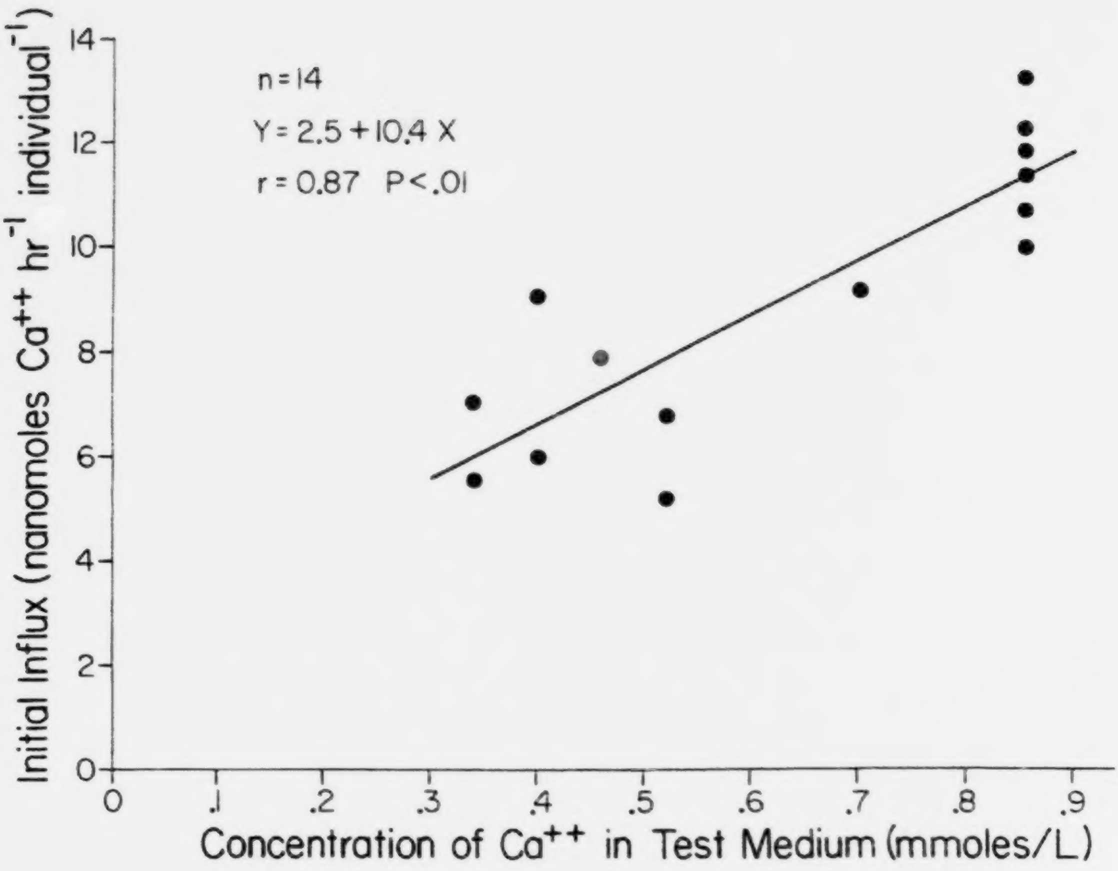

Fig. 2. The relationship between initial influx of Ca^{++} into 14 post-moult *D. magna* and the initial concentration of Ca^{++} in their media.

to take up measurable amounts of ^{45}Ca from the 200 µL media required pooling of 10 to 20 *Daphnia* at the second instar stage. A problem encountered was that frequently several of the animals became entrapped in the surface film of the medium making it difficult to be certain how many in the vial were contributing to the Ca^{++} uptake. Therefore this method was abandoned in favour of use of mature females moulting to release the ephippium since they were larger, could be studied individually and the moment of moulting could be more precisely known.

In laboratory culture, *D. magna* produces ephippia in response to stress, i.e. food shortage, temperature decline, extended darkness or adverse chemical conditions. If food and appropriate environmental conditions are restored, females in culture which have produced ephippia will survive for long periods and go on to produce further parthenogenetic broods (Leonhard and Lawrence, b. in preparation). Thus, we expect that these postmoult females are physiologically typical with respect to Ca^{++} balance.

Influx has been found to depend upon external Ca^{++} concentration in the crayfishes *Austropotamobius pallipes* (Greenway 1974) and *Orconectes virilis* (Malley, unpublished data). The relationship resembles saturation kinetics due to the saturation of the Ca^{++} active transport system at high external Ca^{++} concentrations. To determine this relationship for *Daphnia magna*, a wider range of initial Ca^{++} concentration is required. Nevertheless, the significant correlation between initial influx and initial Ca^{++} concentration in the media indicates that in at least the lower concentrations used here, the Ca^{++} transport system was not saturated. If initial influx is to be reported in the presence and absence of a toxicant, the initial Ca^{++} concentration in the medium must be the same for the determination of b_{normal} and b_{tox}. Further, it is desirable that dry weight be determined on each individual *D. magna* used for Ca^{++} uptake measurements. This will allow the initial influx of each to be expressed on a weight specific basis.

Few techniques are available in the literature which allow repeated estimates on single *Daphnia* of the amount of Ca^{++} taken up from the environment. Porcella et al. (1969) in their study of moulting and calcification in *D. magna* ashed individuals in order to determine the amount of ^{45}Ca taken up over a time interval. For time course measurements, a Ca^{++} analog, ^{85}Sr has been used (Marshall et al. 1964; Porcella et al. 1969). Marshall et al. (1964) removed individuals periodically from the radioactively-labelled medium and counted them live in a well-type NaI scintillation counter. The advantage of our method is the use of liquid scintillation equipment which is more readily available in most laboratories and that Ca^{++} uptake is measured directly rather than by means of an analog which behaves similarly but not identically.

The studies of Marshall et al. (1964) and Porcella et al (1969) show that ^{85}Sr, and by inference Ca^{++}, uptake following moult behaves asymptotically. Uptake is roughly 90% complete within about 15 hours. Toxicity tests completed within six hours of the moult would occur during a time when Ca^{++} uptake is constant. They should not extend much beyond this time. Nevertheless, control

Daphnia not exposed to toxicant must be included in a toxicity test to demonstrate that b_{normal} remained constant over the total experimental period.

D. magna at the first adult instar stage contains an average of 6.7 μg of Ca^{++} (Marshall et al. 1964). If our individuals took up Ca^{++} at a constant rate of 13.1 nanomoles hr^{-1} $individual^{-1}$ for 15 hours, they would accumulate 7.9 μg Ca^{++}. Thus our influx rates are in reasonable agreement with the amount of Ca^{++} that would be expected to be taken up by a larger, older, ephippium-producing female.

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ABSTRACT

Eggs of H. rigida, in two different stages of development, were exposed to methoxychlor and start of hatch, hatch rate, and success of hatch were monitored to determine lethal and sublethal effects. Bioassays were conducted at 24+2°C, at which temperature hatch begins nine to ten days after release from the female. "Early-stage" eggs were exposed to concentrations ranging from 0.09 mg/L to 0.7 mg/L for ten and a half days using Red River water or reconstituted water for incubation and as diluent. In addition, "mid-stage eggs" (fresh and stored) were exposed to similar concentrations using reconstituted water only. Effects ranged from partial suppression of hatch at the lowest concentration to total suppression at the highest concentration, with the incipient LC50 values estimated to be 0.12 mg/L in Red River water and $<$0.09 mg/L in reconstituted water. Mid-stage eggs stored at 8°C for ten weeks were somewhat more tolerant to methoxychlor than fresh mid-stage eggs (estimated incipient LC50 values of 0.07 mg/L and 0.10 mg/L. respectively). Stored eggs may be of value in toxicity testing because thay can be held nearly year round (up to ten months) with minimal maintenance requirements, and are available for toxicity testing on short notice.

Key Words: Eggs, bioassays, methoxychlor, hatching, embryonic development, aquatic, insect

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INTRODUCTION

Toxicity studies on mayflies have been largely confined to the nymphs, probably because this is the most important stage in the aquatic food web. However, all aspects of the animal's life cycle should be considered in order to adequately evaluate the effects of a toxicant on the survival of an organism. McCart et al. (1977) studied the effects of methanol on development and hatching of the eggs of the mayfly Ephemerella sp. and Friesen (in preparation) the effects of saline groundwater on egg viability of the burrowing mayfly Hexagenia rigida (McDunnough). This latter study and a study on the relationship between temperature and duration of egg development of H. rigida (Friesen et al. in press) showed that the egg stage is sensitive to chemical and physical parameters, and that eggs are convenient to use in bioassays. The temperature study also showed that eggs about mid-way through embryonic development could be stored successfully at a low temperature (8°C) for up to ten months: hence, a source of eggs, collected during the summer at peak emergence and mating periods, could be available for use in toxicity testing throughout most of the year.

Methoxychlor, a chlorinated hydrocarbon, used as a larvicide in the control of blackflies and other pests, has been shown to affect non-target organisms, including mayfly nymphs, at treatment levels (Flannagan et al. in press). Literature on the effects of methoxychlor on environmental quality has been reviewed by Gardner and Bailey (1975).

The objective of the present study was to determine the sensitivity of developing eggs of H. rigida to methoxychlor, including eggs which had been stored at 8°C for ten weeks.

MATERIALS AND METHODS

Eggs of ten female imagines collected on 16 July 1978 at the Red River at Winnipeg, Manitoba were dissected into reconstituted water, pooled and aliquots put into acetone-hexane washed 90 mm diam. glass Petri dishes. Incubation temperature during the bioassay was 24 ± 2°C. At this temperature hatch is expected to start about nine to ten days after release of the eggs (Friesen et al. in press). "Early-stage" eggs (just released from the female and in which only yolk is visible) were exposed to nominal methoxychlor concentrations of 0.1, 0.5 and 1.0 mg/L for ten and a half days. Red River water (filtered through 0.22μ filters) or reconstituted water, was used as diluent. Other eggs were allowed to develop for four and a half days in reconstituted water to a "mid-stage" of development (embryonic form just becoming discernible). One batch of dishes with mid-stage eggs (referred to as fresh mid-stage eggs) were exposed to the above concentrations for six days. A second batch was transferred to 8°C. After ten weeks this latter batch of dishes (referred to as stored mid-stage eggs) was transferred to 24 ± 2°C, and after two hours was exposed to the same concentration for seven days.

Eggs were examined for first hatch and for five days thereafter at 12 hour intervals. They were checked once again, about two weeks later when no further hatch was expected (eggs were either deteriorating internally or had turned dark). Embryonic development was noted two times; once four and a half days after eggs were released and later when no further hatch was expected to occur. There were three dishes per treatment with 123 to 836 eggs per dish. Counts were made at x10 magnification and embryonic observations at x25. The reconstituted water, a defined culture medium, was made up from salts dissolved in double distilled water modified from the method described by Provosoli et al. (1970). Chemical characteristics of Red River water and reconstituted water are given in Table 1.

The emulsifiable concentrate from which methoxychlor concentrations were made up contained approximately 20% active ingredient (R. Sebastian personal communication). Initial and final samples (from one dish per concentration)were analysed according to the method described by Solomon and Lockhart (1977). The same methoxychlor solutions used in the early-stage eggs were used for the fresh mid-stage egg exposures, and initial concentrations in the latter case were, unfortunately, not determined.

Criteria used for test measurements were; day of first hatch, hatch rate (cumulative percent hatch of total number of eggs which would finally hatch per concentration per day), and final percent hatch (percent hatch of total number of eggs per concentration). Final percent hatch and standard deviations were calculated using arcsin transformed data. Final percent mortalities due to treatment were calculated using Abbott's formula (Abbott 1925), where adjustments are made for non-treatment related mortality. This is a correction formula commonly used in insect studies when mortality (in this case non-hatch) may be due to factors other than the treatment e.g. unfertilized eggs (Philip Barker personal communication). Incipient LC50's ("the lethal concentration for 50 percent of individuals on long exposure") were estimated graphically by plotting the percent mortality due to treatment versus concentration (see Sprague 1969). Initial concentrations of methoxychlor were used for the estimations.

RESULTS

Initial methoxychlor concentrations were appreciably lower than expected and decreased considerably over the exposure period (especially at the higher concentrations), however, they were initially and remained in a graded series (Table 2). Reduction in methoxychlor levels in the Red River water was considerably higher than in reconstituted water.

Hatch rates did not show considerable differences between treatments and are not shown. Day of first hatch, final percent hatch and standard deviations, and final percent mortality due to treatment are given in Table 3. Hatch did not occur, or was delayed and reduced in varying degrees in the two higher concentrations in all cases. Eggs incubated with Red River water as

Table 1: Chemical characteristics of Red River water (filtered) and reconstituted water.

	Red River Water mg/L	Reconstituted Water mg/L
Cl	35.5	143
SO4	99.0	29.0
Na	31.7	21.6
K	6.6	32.3
Mg	26.3	9.1
Ca	82.6	71.7
Mn	0.02	<0.04
Fe	0.02	<0.04
pH	8.4	6.2
Conductivity	640	610

Table 2: Initial and final methoxychlor concentrations in mg/liter of solutions in which *H. rigida* eggs were exposed continuously in Red River water or reconstituted water, and which fresh and stored mid-stage eggs were exposed in the latter part of embryonic development.

RED RIVER WATER:

Nominal concentrations of methoxychlor in mg/	DAY 0	DAY 10.5
0.1	0.09	0.04
0.5	0.24	0.06
1.0	0.55	0.08

RECONSTITUTED WATER:

Nominal concentrations of methoxychlor in mg/ Continuous Exposure	DAY 0	DAY 10.5
0.1	0.06	0.06
0.5	0.31	0.21
1.0	0.66	0.26
Fresh Mid-Stage Eggs Exposure		DAY 6
0.1	-	0.04
0.5	-	0.22
1.0	-	0.32
Stored Mid-Stage Eggs		DAY 7
0.1	-	0.05
0.5	0.35	0.24
1.0	0.87	0.32

Table 3: Day of first hatch at 24°C, mean and standard deviations of final hatch (of total number of eggs) per treatment, and final percent mortality due to treatment of H. rigida eggs exposed to methoxychlor continuously in Red River water or reconstituted water, and of fresh and stored mid-stage eggs exposed to methoxychlor in reconstituted water.

TREATMENT (3 replicates) Nominal** Concentration Methoxychlor in mg/liter	DAY OF FIRST HATCH (at 24°C)	MEAN AND STANDARD DEVIATION OF FINAL PERCENT HATCH	FINAL PERCENT MORTALITY DUE TO TREATMENT
RED RIVER DILUENT			
continuous exposure;			
control	9.5	42.6±0.01	0.0
0.1	10.0	32.0±1.7	23.8
0.5	10.5	1.0±2.8	93.4
1.0	10.5	0.3±0.8	98.1
RECONSTITUTED WATER			
continuous exposure;			
control	9.5	43.1±0.2	0.0
0.1	9.5	5.7±3.0	82.7
0.5	no hatch	0.0	100.0
1.0	no hatch	0.0	100.0

Continued...

Table 3: (Concluded)

TREATMENT (3 replicates) Nominal** concentration methoxychlor in mg/liter	DAY OF FIRST HATCH (at 24°C)	MEAN AND STANDARD DEVIATION OF FINAL PERCENT HATCH	FINAL PERCENT MORTALITY DUE TO TREATMENT
RECONSTITUTED WATER			
fresh mid-stage eggs exposed;			
control	9.5	41.3+0.2	0.0
0.1	10.0	24.4+4.9	36.3
0.5	no hatch	0.0	100.0
1.0	no hatch	0.0	100.0
stored mid-stage eggs exposed;			
control	10.0	38.1+0.5	0.0
0.1	10.0	21.0+0.4	44.6
0.5	10.5	10.9+7.2	61.6
1.0	11.5	0.3+0.0	99.8

**See Table 2 for actual concentrations

diluent did not seem to be affected as much as eggs incubated with the reconstituted water. Hatching characteristics of eggs exposed continuously to methoxychlor were similar to mid-stage eggs (exposed only in latter part of development) except that there was a higher mortality at 0.1 mg/L. Stored mid-stage eggs seemed to be somewhat less sensitive to methoxychlor than fresh mid-stage eggs.

When embryonic development was observed on day four and a half, eggs (in a proportion similar to that in the controls) were at similar stages of development in all treatments. By the later observation time, development had ceased in eggs (which had shown some development but had not hatched) at advanced stages of development in all concentrations with treatment related mortality (whether eggs had been exposed continuously or only in the latter period of development).

The estimated incipient LC50 values for eggs exposed to methoxychlor continuously with Red River was 0.12 mg/L, with artificial water was <0.06 mg/L, for fresh mid-stage eggs was 0.07 mg/L, and for stored mid-stage eggs was 0.10 mg/L (Fig. 1).

DISCUSSION

The initial concentrations of methoxychlor were lower than expected probably because of adsorption of methoxychlor to the glass surfaces of the preparation containers and Petri dishes (J. Solomon personal communication). The lower methoxychlor levels found in Red River water compared to the reconstituted water may have been due to the adsorption of methoxychlor onto extremely fine particulate matter. Merna and Eisele (1973) in a laboratory experiment on breakdown rate of methoxychlor in water suggested that the initial high disappearance rate which they observed may have been due to adsorption of methoxychlor onto particles which settled out and were consequently missed in the sampling procedure.

Hatching characteristics of eggs exposed continuously to methoxychlor and those exposed only in the latter period of development were similar. In the temperature study (Friesen et al. in press) and the saline groundwater study (Friesen in preparation) early-stage eggs showed a greater sensitivity than did mid-stage eggs. The observations of embryos in the present study indicated that the most sensitive period to methoxychlor occurred in advanced stages of development and perhaps methoxychlor, which seems to act on the nervous system (see Gardner and Baily 1975), has no target tissue until the nervous system of the embryo becomes developed. Although eggs were exposed to methoxychlor not less than six days the sensitive period may be much shorter and it would be important to know when the target tissue is developed sufficiently to be affected and determine exposure times with no adverse effects.

The estimated LC50's are higher than proposed levels for the Great Lakes (0.04 mg/L) (Great Lakes Quality Board 1976) but are lower than maximum levels (0.3 mg/L to 0.4 mg/L) used in the treatment of some rivers for blackflies (Fredeen 1974). These LC50 values, particularly for the Red River water,

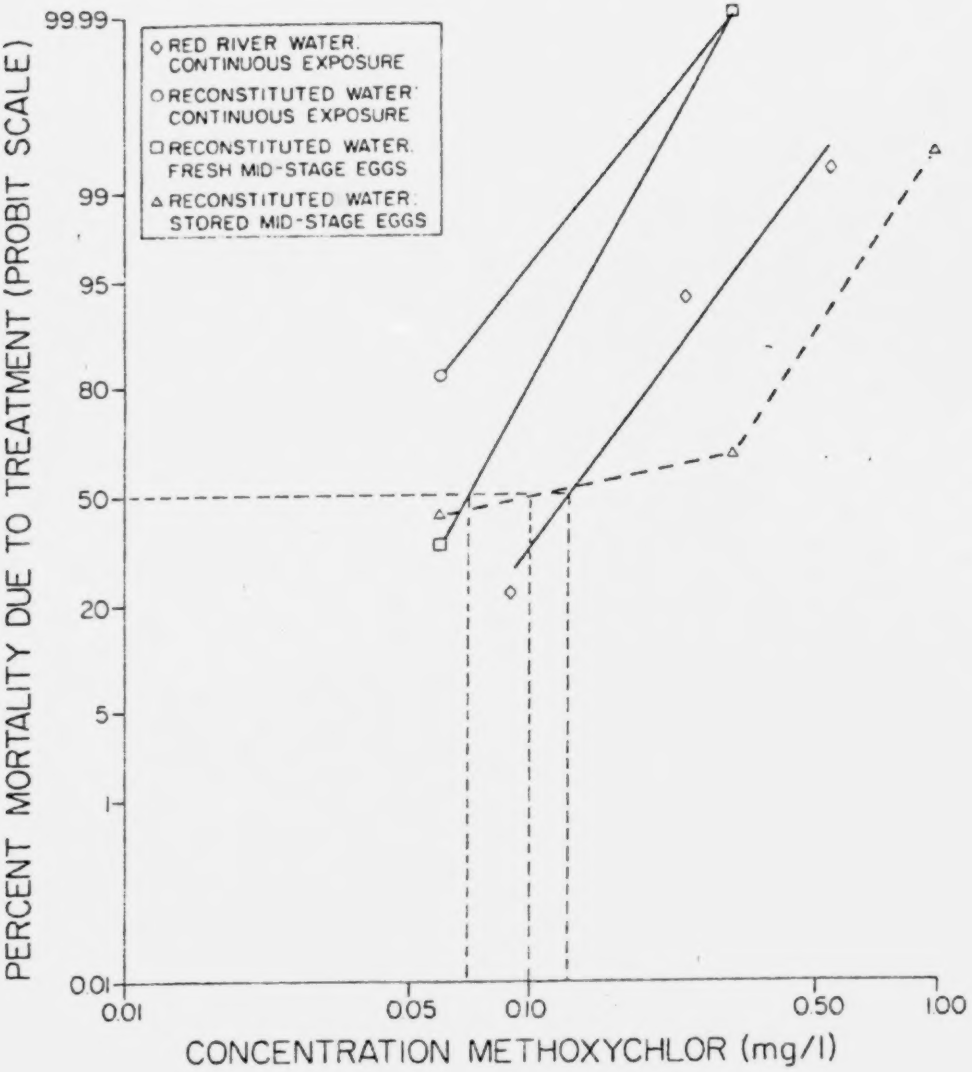

Fig. 1. Estimation of incipient LC50's (smaller dotted lines) of *H. rigida* eggs exposed to methoxychlor: final percent mortality due to treatment on probit scale versus concentration of methoxychlor on log scale.

are conservative values since they were estimated using the initial concentration levels which decreased considerably over the exposure period and were probably lowest during the most sensitive period of development. Although field conditions are obviously different from this laboratory study, the estimated LC50 values are near enough to levels which may and do occur in the aquatic environment that possible adverse effects of methoxychlor on the egg stage cannot be eliminated. Information on the relative tolerance of the nymphal stage is needed to adequately evaluate the toxic effects of methoxychlor on this organism.

The reason for the apparent decreased sensitivity in stored mid-stage eggs is not known. Clubb et al. (1975) found that the stonefly nymphs Arcynopteryx collected after November were more tolerant to cadmium than those collected prior to November and suggested that this might have been due to the insects being in a more advanced stage of development. This is probably not the case here. The study done by Friesen et al. (in press) indicates that development does not occur at 8°C. This is also supported in the present study since stored mid-stage eggs took half a day longer to hatch than did fresh mid-stage eggs when total time at 24°C is considered.

This study(and the saline groundwater study (Friesen in preparation)) has shown that eggs are sensitive to chemicals and are convenient to work with as bioassay material. Although results of stored mid-stage eggs may not necessarily be extrapolated to a field situation, eggs held in this manner may prove useful as a screening test in the evaluation of the toxicity of a chemical because of ease in maintenance over prolonged periods and availability on short notice.

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ABSTRACT

Tubificid oligochaetes, particularly the resistant species Limnodrilus hoffmeisteri Claparède and Tubifex tubifex (Müller) have long been used as indicators of organic pollution; however, their tolerance to heavy metals has not been established. The first part of this paper, which reviews the literature, indicates that tubificid oligochaetes are more tolerant of certain heavy metals than associated benthic animals such as chironomids.

The second part of this paper documents the use of tubificids as indicators of biologically available heavy metals in sediments in a recent study of the Fraser River, B.C., undertaken by the Inland Waters Directorate. During the course of this study, monthly measurements were made of nine heavy metals (Cu, Zn, Pb, Fe, Mn, Ni, Co, Cd and Hg) in tubificids and in the sediments from which they were collected. The tubificids L. hoffmeisteri and T. tubifex were used because they were the only benthic organisms consistently present in sufficient numbers for metals analyses. Data from this and other studies are presented to illustrate the use of tubificids in assessing biological accumulation of heavy metals and their use as a monitoring tool to detect future changes in metal levels. Methodology is discussed, including sampling techniques, sources of contamination, the use of preservatives, and identification of tubificids.

The third part of this paper considers the use of tubificids as a monitoring tool for heavy metal levels in marine, estuarine and fresh water situations. Discussions include the use of benthos as indicators of biologically available metals in sediment, the position of tubificids as a link in the food chain between bacteria and fish, and the cosmopolitan distribution of such tubificids as L. hoffmeisteri and T. tubifex.

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INTRODUCTION

Oligochates are small, segmented, hermaphroditic annelids found in freshwater and marine habitats throughout the world. The family Tubificidae consists of 26 genera, many of which are cosmopolitan. Members of this family have been used extensively as indicators of organic pollution (Brinkhurst & Jamieson, 1971), however oligochates as a group have often been considered to be intolerant of heavy metals (Aston, 1973). Hynes (1960) suggested using oligochaetes as indicators of heavy metal pollution by noting their absence as compared to the presence of more tolerant species. Several recent studies (Whitley and Sikora, 1970 and Brkovic-Popovic and Popovic, 1977 a,b) have indicated that tubificids are tolerant to certain metals in solution. The purpose of this paper is to review the literature on metal uptake by tubificids, and to document their use as monitors of heavy metal levels through tissue analysis. The tubificid species, *Limnodrilus hoffmeisteri* Claparéde and *Tubifex tubifex* (Müller), often the sole surviving benthic organisms in organically polluted environments, will be shown to be of particular value in this regard.

The misconception about tolerances of oligochaetes to heavy metals dates from 1938 when Jones noted, in laboratory experiments, that *T. tubifex* was killed by combined concentrations of copper and lead in water equivalent to over 1,000 mg liter^{-1}. Several subsequent studies, all involving copper, supported the premise of oligochaete intolerance to high metal levels. During a field survey, Butcher (1946) observed that copper from effluent (receiving water concentrations of 0.12-1.2 mg liter^{-1}) eliminated tubificid populations and most of the benthos. Learner & Edwards (1963) noted, in laboratory experiments, that *Nais* spp. (family Naididae) were killed within 6 hours by 1 mg liter^{-1} of $CuSO_4$.

Whitley's (1967) and Whitley and Sikora's (1970) laboratory experiments were the first to show that tubificids have high tolerances to solutions of some heavy metals. These investigators noted high tolerances of *L. hoffmeisteri* and *T. tubifex* to lead and zinc in laboratory experiments. Death finally resulted from precipitation of the worms' mucous coating, which inhibited epidermal respiration.

More recent laboratory studies have been done by Brkovic-Popovic and Popovic (1977 a,b) who studied the respiration rate and survival of *T. tubifex* exposed to solutions of copper, cadmium, mercury, zinc, chromium and nickel. Results indicated that, with the exception of mercury, toxicity depended on hardness and alkalinity. The range of 48 hour LC50s in mg liter^{-1} were: Cu, 0.006-0.89; zinc, 0.11-60.2; chormium, 0.06-4.57; nichel, 0.08-61.4; cadmium, 0.03-0.72; and mercury, 0.06-0.10. No mortalities occurred in the controls.

Thus oligochates have been shown to be particularly sensitive to certain metals in solution (eg. Cu, Cd and Hg) and tolerant of others (eg. Zn, Cr. Ni, and Pb). However, aquatic oligochaetes are infaunal and the chemical and physical conditions in sediments may be very different from those in the overlying water column (Chapman & Brinkhurst, 1978). Therefore oligochaete tolerances to heavy metals should be judged on the basis of metal levels in sediments and interstitial waters, rather than on levels in the water column.

Recent field studies of heavy metal levels in sediments containing populations of tubificid oligochaetes suggest that these animals have a high tolerance to sediment metal levels. Funk et al (1973) noted that zinc concentrations of 1,000-7,000 µg g^{-1} dry weight in bottom sediments (as determined by nitric-perchloric acid extraction) did not affect the distribution of oligochaetes in an urban stream. Wentsel et al (1977 a) measured metal levels in Palestine Lake, Indiana by nitric-perchloric extraction and found that Limnodrilus spp. survived despite cadmium, zinc, and chromium levels (in µg g^{-1} dry weight) of, respectively, 970, 14,00 and 2,100. These levels eliminated the midge Chironomus tentans and most of the rest of the benthos.

Information to date on tubificid tolerances to heavy metals in sediments is summarized in Table 1. This figure combines the results of several field surveys and records maximal sediment metal levels noted to contain tubificid populations. These numbers do not necessarily represent the highest metal levels that can be tolerated by tubificids, and it should be emphasised that metal levels vary with extraction technique. However, these data indicate that tubificid oligochaetes can tolerate high levels of certain heavy metals in sediments.

METHODS

This section describes the methods of sampling, pretreatment and analysis used to measure heavy metals in tubificids collected from the Lower Fraser River, B.C. Suggestions are made for improvements to currently used sampling techniques.

This study measured monthly levels of nine heavy metals (Cu, Zn, Pb, Fe, Mn, Ni, Co, Cd and Hg) in benthic animals and in the sediments from which they were collected. The complete results of this investigation , which was carried out from September 1977 · September 1978 will be the subject of a future publication. The data from six months of sampling are shown in the present paper.

Field Sampling

Sampling sites were chosen, after a preliminary survey, to provide sufficient biomass of animals for anlaysis. The lower Fraser River is highly industrialized and it was thus difficult to locate sampling sites which were not influenced by local sources of metal contamination (eg. storm sewers, small boat docks, municipal and industrial effluents). Additionally, sampling sites were chosen with consideration to such logistic problems as access, the location of future dredging operations and the location of shiftin log booms.

TABLE 1 MAXIMAL SEDIMENT HEAVY METAL LEVELS OBSERVED TO CONTAIN POPULATIONS OF TUBIFICIDS

(Values are in μg g^{-1} dry weight)

METAL	LEVEL	SPECIES	REFERENCE	SEDIMENT EXTRACTION METHOD
Cu	56	*L. hoffmeisteri* and *T. tubifex*	Bindra and Hall, 1978	peroxide-nitric acid
Pb	204			
Fe	24,000			
Mn	610			
Ni	33	*L. hoffmeisteri* and *T. tubifex*	data from current Fraser River study	peroxide-nitric acid
Co	11			
Cr	2,100	*Limnodrilus* sp.	Wentsel *et al*, 1977a	nitric-perchloric acid
Zn	14,000			
Cd	970			

Sediments were sampled with a Ponar grab sampler, and care was taken to exclude sediment which had contacted the sides of the grab. A benthic sampler composed of inert material would be a useful development for future benthic metal studies. In shallower water, sampling could be done by digging into the substrate with plastic instruments.

Once sediment was collected, it was handled with clean plastic instruments and stored in clean plastic buckets. The sediment was screened on site through a 0.5 mm mesh sieve, 30 cm in diameter, using water from the collection site. A stainless steel sieve was chosen as a compromise between durability and contamination problems; these sieves can be made to order in various diameters. Sieves made of inert plastic materials were too fragile for sustained field use. The 0.5 mm mesh is ideal for retaining tubificids while removing fine sediment particles that complicate sorting.

The amount of sediment collected depended on the biomass of worms present; in the Fraser River 4-7 full (0.05 m^2) Ponar grabs were necessary. This involved several hours of work for one person at each sampling site.

Pretreatment

After sieving, samples were transported to the laboratory as soon as possible. All samples were sorted within 24 hours of collection because changes in tissue heavy metal levels may occur with storage time (Jones, 1978). Sorting was done against a dark background in nitric acid cleaned glass pans using deionized, distilled water. Electron microscope stainless steel forceps were use for picking out the worms as teflon-coated forceps were not fine enough.

Tubificids are often covered with mucous secretions which collect sediment and therefore should be removed. This was most easily done by wearing surgical silicone rubber gloves (washed in deionized, distilled water), and drawing the worms across this surface. The problems of contamination during sorting were examined by an experiment in which two sieved samples were divided into halves, one half being sorted with bare hands and the other with gloves. The use of gloves decreased tissue metal levels and gloves were used in all future manipulations.

Following cleaning, tubificids were put individually through two washes of deionized, distilled water and, when sufficient biomass was available for analysis, frozen at -20°C. The samples to be analyzed for mercury were frozen until analysis, while samples to be analyzed for the other metals were freeze-dried.

In other published studies, tubificids were preserved in formal prior to analysis. However, our studies have shown that the use of formalin leads to an overestimation of metal levels. Several samples of tubificids were collected at different times. These samples were then subdivided, part of the sample was sorted alive, and the other part preserved in 10% formalin prior to sorting. Sorting techniques in both cases followed the methods outlined above.

Table 2 shows a comparison of metal levels in tubificids sorted from live and preserved material. Sample A was divided into four parts after sieving, with three parts preserved in formalin before sorting and one part sorted alive. Samples B-E were divided into two parts, half sorted after preservation and half sorted alive. Most tissue metal levels, especially copper and manganese, show increases as a result of using formalin (except Co and Cd, where measurements approach detection limits). We therefore suggest that, in future investigations, tubificid tissue should not be preserved in formalin prior to metals analysis.

During sorting, oligochaetes can, with a little practice, be sorted visually to family so that samples for analysis contain only Tubificidae. In order to determine species composition, a representative sub-sample of 50 to 100 worms can be removed during sorting, preserved in formalin and then mounted on slides for identification using Amman's lactophenol as the clearing agent (40 g carbolic acid, 40 ml lactic acid, 80 ml glycerol and 40 ml water). Brinkhurst & Jamieson's taxonomic revision of the Oligochaeta (1971) may be used to verify identifications, however regional keys may also be available. In the Fraser River study, only two species of tubificids were present in significant numbers: L. hoffmeisteri and T. tubifex. These two species are the most common cosmopolitan tubificids, being found in great numbers in lakes and rivers throughout the world (Brinkhurst & Jamieson, 1971).

Analysis

Tissue metal levels as represented in Tables 2 and 3 were digested by the same technique: peroxide-nitric acid extraction (4 ml of 30% H_2O_2, 1 ml HNO_3 extracted at medium heat for 2½ hours). For all metals except mercury, a dry weight of 0.125 g was required and measurements were done using a flame atomic absorption spectrophotometer. The possibility of using a flameless absorption spectrophotometer for certain metals should be investigated as this technique requires a smaller sample and could cut down on sampling time. Various techniques have been used in other studies for analysis of tissue heavy metal levels. Although most techniques depend on an initial wet or dry oxidation step to release the metals, there are no standard methods of analysis.

For the Fraser River study, a peroxide-nitric acid extraction was used to measure tissue levels of all metals except mercury. This technique was used in order to compare results with an earlier study on the oligochaete metal levels in the watersheds of the Fraser River (Bindra and Hall, 1978). To determine how this method

TABLE 2 THE EFFECTS OF FORMALIN ON METAL LEVELS IN TUBIFICIDS: COMPARISON OF TUBIFICIDS SORTED FROM LIVE AND PRESERVED MATERIAL (Values are in μg g^{-1} dry weight. Extraction is by peroxide-nitric acid)

METAL	SAMPLE A		SAMPLE B		SAMPLE C		SAMPLE D		SAMPLE E		$\overline{X}$% increase and 95% C.L. for increase in metal levels due to formalin
	PRESERVED	LIVE	PRESERVED	LIVE	PRESERVED	LIVE	PRESERVED	LIVE	PRESERVED	LIVE	
Cu	91±20*	28	35	14	102	24	67	20	39	27	186 ± 50
Zn	180±25	110	180	85	240	110	130	110	120	120	62 ± 24
Pb	<10	6	12	10	30	23	15	17	47	34	25 ± 21
Fe	5,300±860	3,900	5,500	2,700	9,100	6,300	7,500	4.700	4,800	3,200	79 ± 33
Mn	323±29	55	290	51	420	120	240	96	200	73	306 ± 67
Ni	15± 6	9	18	<1	68	21	21	13	4	6	73 ± 20
Co	<4	6	<2	<1	<8	<4	<4	<4	<1	3	} below } detection } limits
Cd	<2	<2	<2	<1	<8	<1	<2	<2	<2	<1	

* means of 3 replicates ± one standard deviation

compared with other techniques, tubificid tissue samples were analyzed by four different methods of extraction: a peroxide-nitric acid digestion, a 24 hour hydrogen peroxide digestion, a 66 hour hydrogen peroxide digestion, and a nitric hydrochloric acid perchloric acid digestion, (5 ml HNO_3, 5 ml HCl_3, 2 ml $HClO_4$ extracted at medium heat for 1 hour). The results shown in Table 3 are not replicated and only provide a rough comparison: however, the values for Cu, Zn and Mn seem comparable between the different extraction methods. Lead and iron are low for the peroxide 24 hour extraction, although when extraction proceeded for 66 hours, the readings were much higher than by the other methods. Iron values are variable. Ni, Co and Cd all approach detection limits and are very similar except in the case of the 66 hour peroxide extraction. These variations point out the need for a standard technique that will allow comparisons to be made between different studies. Published reports of tissue heavy metal levels in tubificids use either the peroxide-nitric acid or a nitric perchloric extraction. Therefore, for the purposes of standardization and comparison, it is recommended that one of these techniques be chosen for future metal studies in tubificids. Also, all analyses should be run using NBS standards to ensure close to one hundred percent metal recovery.

Mercury was measured in the present study using a wet digestion followed by a cold vapour technique (DOE Analytical Manual, 1974). The initial digestion may be done by various methods, and standardization is also recommended. In this study, a dry weight of 0.125 g was required for mercury analysis. The sample was oven-dried at 50°C prior to analysis.

A final point concerning metals analysis of tubificids is that of gut sediment analysis. Allowing tubificids to evacuate their guts is meaningless without taking steps to separate out the feces, because coprophagy is common in these worms (Brinkhurst, 1973). In the Fraser River study, measurements of metal levels in tubificids were corrected for gut contents. This was done by collecting undigested sediment from the peroxide-nitric acid digestion of tubificid tissue on pre-weighed, acid-washed (0.2% HNO_3) 0.45μ SARTORIUS membrane filters. The filters and sediment were dried to constant weight and weighed. The metal levels in the sediment which the tubificids inhabited were measured, and used to calculate gut sediment levels. These values were then subtracted from measured tubificid tissue metal levels. However, in the present paper, uncorrected values are shown for the purpose of comparison with other studies.

McNurney (1977) found no statistical difference between lead levels in tubificids will full and evacuated guts. However, in the present study, metals in sediment gut contents could represent a substantial portion of total tissue metal levels. This is illustrated in Table 4 which combines data on seven metals from a year of monthly sampling at two stations in the lower Fraser River. Although gut sediment weight averages 14.9% of total tubificid weight, the metal load contributed varies from a mean value of 26.0% for zinc to a mean value of 73.0% for nickel. These values will vary depending on method of digestion and sediment metal levels and therefore we suggest that future investigations carry out a gut sediment correction.

TABLE 3 COMPARISON OF DIFFERENT EXTRACTION METHODS FOR TUBIFICIDS

(Values are in $\mu g\ g^{-1}$ dry weight)

	SAMPLE A			SAMPLE B			
METAL	H_2O_2/HNO_3	H_2O_2 24 hours	$HNO_3/HCl/HCLO_4$	H_2O_2/HNO_3	H_2O_2 24 hours	$HNO_3/HCl/HClO_4$	H_2O_2 66 hours
Cu	20	24	23	15	18	23	20
Zn	110	120	120	160	200	230	220
Pb	18	10	19	62	18	36	100
Fe	4700	470	8100	2600	330	880	5400
Mn	96	63	130	70	74	73	120
Ni	13	<4	19	<4	<4	<4	15
Co	<4	<4	6	<4	<4	<4	7
Cd	<2	<2	<2	<2	<2	<2	<2

TABLE 4. THE CONTRIBUTION OF GUT SEDIMENT CONTENTS TO TISSUE METAL LEVELS IN TUBIFICIDS

(Extraction method is peroxide-nitric acid. Metals extracted from gut sediments are shown as a percentage of total tissue metal levels.)

METAL	Mean percent and 95% confidence limits	Sample size
Cu	31 ± 6	23
Zn	26 ± 5	23
Pb	61 ± 10	22
Fe	72 ± 10	23
Mn	70 ± 10	23
Ni	73 ± 10	20
Co	55 ± 26	8

(mean weight of gut sediment and 95% C.L. = 14.9 ± 3.0, n=23)

DISCUSSION

Three reservoirs for metals exist in the aquatic environment: water, sediment and biota. Renfro (1973) indicates that sediments are the major repository, sometimes holding over 99% of the metals present. The monitoring of metal concentrations in sediment has two main advantages over measurements in the water column (Phillips, 1977): concentrations in sediments are three orders of magnitude higher than in the water thus reducing problems of analysis and contamination, and sediments may integrated environmental fluctuations of heavy metal levels. However, monitoring of heavy metal levels in biota has both these advantages and additionally provides information on the biological availability of heavy metals.

Published measurements of heavy metals in tubificids are summarized in Table 5, which shows that levels of metals in tubificids as compared to sediments are variable in all geographic locations studied. Mathis & Cummings (1973) found that, except for iron and manganese which were lower and copper which was slightly higher, metal levels in tubificid tissues were similar to those in sediments. Bindra & Hall (1978), however, noted a significant variation in accumulation at different sites in the lower Fraser River. The trend was for copper, zinc, lead and manganese to be accumulated above sediment levels. In Rhodesia (Greichus *et al*, 1977), mercury was not accumulated by *Branchiura sowerbyi* despite sediment mercury levels almost three times those at New Westminster on the Fraser. To explain variations in bioaccumulation, Bindra & Hall (1978) state that trace metals in benthic organisms are not always highest in areas of the highest total sediment trace metal concentrations. They relate biological availability to a number of factors: the geochemical phases of the metal, organic content of sediments, Eh, pH and sulphide levels. This points out the importance of measuring heavy metal levels in such benthic animals as tubificids as well as in sediments in order to estimate the biological availability of the heavy metals present.

Infaunal benthic organisms such as tubificids are of particular importance in monitoring toxic substances due to their lack of mobility; i.e., unlike pelagic organisms, they reflect conditions at a specific location. Because they live in sediments, which are the major repository for metals, benthic populations also reflect the long-term impact of metals on the ecosystem (Renfro, 1973).

During our Fraser River study, only three groups of benthic organisms were collected in sufficient numbers from the benthos for tissue metal analysis: tubificids, chironomid larvae and ammocoetes (lamprey) larvae. However, only tubificids were consistentl present in sufficient biomass for monthly tissue heavy metal analys Measurements carried out over a full year indicated no significant variations in heavy metal levels in sediments or in tubificids, although there was some variation observed in ammocoetes larvae. Seasonal changes in metal levels in certain organisms have been observed as a result of biological cycles. For example, seasonal changes in copper concentrations in the whelk *Busycon canaliculatum* are associated with an annual cycle of behavioural and physiologica

TABLE 5 COMPARISON OF MEAN METAL LEVELS IN TUBIFICIDS IN DIFFERENT STUDIES AND DIFFERENT GEOGRAPHICAL AREAS
(Values are in μg g^{-1} dry weight. Sediment metal levels, in μg g^{-1} dry weight are indicated in parentheses under the tissue values. Asterisks indicate accumulation above sediment levels.)

SPECIES	Cu	Zn	Pb	Fe	Mn	Ni	Co	Cd	Hg	REFERENCE	DIGESTION	PRESERVATION TECHNIQUE	AREA	
L. hoffmeisteri and T. tubifex	23* (19)	41 (81)	17 (28)			11 (27)	1.6 (6)	1.1 (2)		Mathis and Cummings, 1973	nitric-perchloric acid	alcohol	Illinois River	
L. hoffmeisteri and T. tubifex	119* (56)	439* (104)	486* (204)	13450 (24000)	863* (570)					Bindra and Hall, 1978	peroxide-nitric acid	formalin	Brunette River	Fraser River Watershed
	33* (17)	289* (62)	19 (39)	8900 (22400)	800* (610)								Salmon River	
	34 (53)	87 (150)	6 (28)										Ladner Channel	
B. sowerbyi and others	72* (38)	130* (100)	1 (41)		28 (350)			0.05 (0.4)	0.08 (0.28)	Greichus et al, 1977	nitric-perchloric acid and total Hg	formalin	Lake McIlwaine, Rhodesia	
L. hoffmeisteri and L. tubifex	25 (29)	159 (181)	58 (130)	5517 (11400)	137 (313)	<11 (30)	<2 (11)	<3 -	0.75* (0.10)	Current study	peroxide-nitric acid and total Hg	none	New Westminster	Fraser River
	14 (31)	88 (105)	12 (38)	2633 (13076)	56 (307)	6 (33)	<3 (11)	<2 -	0.12* (0.07)				Oak Street	

change (Betzer & Pilson, 1975). The lack of seasonal change in tubificids is a factor in favour of using them to monitor the biological availability of heavy metals.

Although ammocoetes larvae are an attractive subject for metal analysis due to their large size, they were not used in the Fraser River study for two reasons: they can swim and therefore metal levels in their tissues may not be representative of the area from which they are collected, and they cannot always be collected consistently. Although chironomid larvae are an important food source for salmon fry and other fish (Northcote, 1976), they are less tolerant of sediment metal levels than are tubificids (Wentsel *et al*, 1977a and Bindra & Hall, 1978) and have been noted to avoid sediments with high metal levels (Wentsel *et al*, 1977b). Also, chironomid taxonomy is more difficult than tubificid taxonomy, as speciation can only be done accurately by raising the larvae to emergence. Chironomids were consistently present in sediments sampled on the Fraser River, but rarely presen in sufficient numbers for heavy metals analysis. A final problem with using chironomids as indicators is that of determining specifi metals uptake routes. Chironomids can feed herbivorously, detrital or carnivorously on oligochaetes, especially *L. hoffmeisteri* and *T. tubifex* (Loden, 1974). It is therefore difficult to determine what portion of heavy metals in chironomid tissues derives from the sediments they live in.

Tubificids are suitable benthic animals to use as monitors of heavy metal pollution in the Fraser River and similar aquatic environments for a number of reasons:

i. they are part of the benthos, are long lived (2-3 years) and therefore integrate sediment concentrations over time

ii. they are often the most abundant organisms in muddy and organically polluted substrates and provide sufficient biomass for analysis

iii. they are easy to sample and are hardy enough for laboratory experiments

iv. they tolerate wide ranges of temperature and salinity (Brinkhurst, 1973) and can therefore be found in a variety of environments.

The problem of determining uptake routes of heavy metals is simpler for tubificids than for many other species. Three pathways are possible in tubificids: by ingestion of bacteria which have accumulated metals from sediments, by ingestion of sediment particles containing metals, and by uptake from solution, i.e. the water column or interstitial water. Patrick & Loutit (1976) demonstrated passage of metals from bacteria to tubificids for copper, manganese, iron, lead and zinc and noted upper concentration values in the worms (in $\mu g\ g^{-1}$ dry weight) of, respectively, 620, 25, 1955, 568 and 868. However, accumulation can also proceed from solution.

Dean (1974) noted that unidentified tubificids accumulated ^{65}Zn from water but not from sediment. D'Angelo & Signonle (1974) found uptake of mercury by T. tubifex was via the gut at low concentrations (0.1 mg liter^{-1}) and by diffusion across exposed body surfaces at higher concentrations(0.3 mg liter^{-1}); however the latter concentration probably represents a toxic dose (Brkovic-Popovic & Popovic, 1977).

The problem of determining what portion of heavy metal levels measured in tubificids is absorbed from solution can be indirectly resolved in field studies by measurements of metal concentrations in interstitial water and in the water column. In the Fraser River, metal levels in the water column and in interstitial water are three orders of magnitude less than in the sediments. This suggests that accumulation of metals by tubificids in the Fraser River is probably by ingestion of bacteria and sediments.

Other factors supporting the use of tubificids as monitors of heavy metal levels include the fact that they form an important link between bacteria and fish. Patrick & Loutit (1976 & 1978) have shown that tubificids accumulate the metals Cr, Cu, Mn, Fe, Pb and Zn from bacteria in sediments and that these metals can be passed on in significant amounts to fish feeding on tubificids. Tubificids have been shown to be available to bottom-feeding fish and may be an important food source (Milbrink, 1973).

Although individual species of tubificids were not separated for analysis, there was no indication in the Fraser River study that seasonal variations in species composition had any effect on metal levels. This is supported in laboratory experiments by Whitley & Sikora (1970) who noted similar LD50s and physiological reactions to heavy metals in water by L. hoffmeisteri and T. tubifex. In our Fraser River study, the species composition of the samples was known, and it was considered unnecessary to analyze individual species of tubificids.

Finally, the use of tubificids to monitor heavy metal levels may result in a desirable standardization of heavy metal studies of the benthos as species of tubificid oligochaetes are found in freshwater, estuaring and marine environments. In particular, L. hoffmeisteri and T. tubifex can be used to monitor metals in most freshwater and estuarine environments. These two species are cosmopolitan, occurring together in high numbers in organically polluted environments (Brinkhurst & Jamieson, 1971). Because of their tolerance to salinity, and because of the modifying effects of sedimentary habitats (Chapman and Brinkhurst, 1978), these species can be used to make comparisons between sites with different salinity regimes. In summary, tubificids possess many of the qualities required to monitor metals in the benthic environment.

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McLeese[*], D.W., D.S. Pezzack[**], and C.D. Metcalfe[*]. 1979. Uptake of PCB's from sandy sediments by *Nereis virens* and *Crangon septemspinosa*. Proc. Fifth Annual Aquatic Toxicity Workshop, Hamilton, Ontario, Nov. 7-9, 1978. Fish. Mar. Serv. Tech. Rep. 862, pp. 295-296.

EXPANDED ABSTRACT

Behaviour Observations *Nereis* (marine worms) left sediment or protruded from their burrows with sediment containing 0.5 ppm PCB's or more. They were sluggish and excreted large amounts of mucus, but returned to the sediment after 4-5 days. *Mya* (clams) left the sediment and were sluggish with sediment containing 1.0 ppm PCB. They did not rebury.

Initial Uptake Observations *Nereis* exposed to sediment spiked at 1 ppm PCB's accumulated PCB's during 50 days' exposure with no indication of achieving an equilibrium concentration (maximum accumulated 7.8 ppm).

Mya showed an initial uptake during 48 hr attributed mainly to uptake from suspended fine sediment particles (maximum accumulated 0.25 ppm) with no further increase up to 50 days.

Mytilus (mussel) exposed to water from above tests showed no change in PCB content during 50 days.

The uptake of PCB's seems to be related to the degree of contact between the animal and the sediment, i.e., uptake by *Nereis* $>$ than by clams $>$ than by mussels.

PCB Uptake by *Nereis* Testing was refined to examine relationships between uptake and time, uptake and worm size, uptake and PCB concentration in the sediments.

(a) Uptake and time: 3-4 gm worms exposed to sediment with 0.58 ppm Aroclor 1254 accumulated the PCB to 3.5 ppm during 32 days with no indication of reaching an equilibrium concentration (line equation $Y = 0.004X(hr) + 0.394$, $r = 0.98$). Results were similar to initial results mentioned above.

(b) Uptake and worm size: Worms ranging in weight from 0.5-4.5 gm and exposed to 0.17 ppm PCB for 32 days showed that smaller worms accumulated more PCB than larger ones (line equation $LnY - 0.336 - 0.477 \ln X$, $r = 0.96$). A similar trend exists for oxygen consumption rate and worm size. Concentration factors ranged from 10.8 for 0.6 gm worms to 3.8 for 4.7 gm worms.

(c) Uptake and sediment concentration: Tests with uniform sized worms exposed to sediments with 0.03, 0.08, 0.17 and 0.58 ppm PCB showed a direct straight line relationship between PCB concentration in sediment and in worm. Those exposed to 0.03 ppm contained 0.1 ppm, those exposed to 0.58 ppm contained 3.5 ppm at 32 days.

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Excretion of PCB by *Nereis* *Nereis* were exposed to PCB in water for initial loading then placed in clean sediment to follow excretion rates. Large worms (7-8 gm) containing 0.25 and 0.75 ppm PCB showed little or no excretion during 20 days. Those with 1.5 ppm PCB showed a slight drop after day 4 with no further change to 35 days. Small worms (3 gm) with initial concentration of 1.75 ppm PCB also showed a slight drop in concentration after day 4. These data indicate that excretion of PCB's from *Nereis* is relatively slow.

PCB Uptake from Sediment and Water *Nereis* accumulated PCB's from water much more rapidly than from sediment. Concentration in a 2-gm worm in sediment with 0.03 ppm PCB was 0.13 ppm by 32 days (C.F. 4.3X or 0.14X/day). Concentration in a 3-gm worm in water with 0.003 ppm PCB was 1.75 ppm in 1 day (C.F. of 530X/day). Other similar examples show a much faster uptake of PCB from water than from sediment.

Uptake of PCB by *Crangon* *Crangon* ranging in weight from 0.1 to 3 gm and exposed to sediment with 0.13 ppm PCB for 32 days showed that smaller ones accumulated more per unit weight than the larger ones (line equation $Y = 0.487 - 0.089X$, $r = 0.96$). Concentration factors ranged from 3.5 for 0.1 g shrimp to 1.9 for 3 g shrimp, and are somewhat lower than the factors for *Nereis*.

Food Chain Accumulation Potential Lobsters were fed spiked mussels at 5 and 50 ppm nominal concentration (1.7 and 16.8 ppm measured) for 6 weeks at the rate of 10 g/week (450 gm lobster); followed by 6 weeks of feeding with uncontaminated food. PCB concentration in the lobster hepatopancreas increased during the feeding with spiked food reaching about 10 ppm in those getting the lower content food and reaching 40 ppm with the higher content food. When fed clean food, the concentration of PCB in hepatopancreas decreased slowly.

Summary PCB's accumulated most readily from sediment by small active animals and by those species which are in direct contact with the sediment. Decreased uptake with increasing animal size may be related to metabolic rate or more simply to relative surface area or both. Once accumulated, PCB's appear to be retained in the animals for considerable periods. A slow rate of excretion is consistent with the observation that equilibrium concentrations were not detected within the experimental periods among animals exposed to contaminated sediments. The concentration factors for sediment exposures are quite low both for *Nereis* and *Crangon*. There is a potential for accumulation and transfer through the food chain since lobsters fed contaminated food accumulated PCB's in the hepatopancreas and exhibited a relatively slow rate of excretion.

Lee*, K., C. Nalewajko and T.R. Jack. 1979. Effects of vanadium on freshwater algae. Proc. Fifth Annual Aquatic Toxicity Workshop, Hamilton, Ontario, Nov. 7-9, 1978. Fish. Mar. Serv. Tech. Rep. 862, pp. 297-310.

ABSTRACT

1. Field studies in Lake Erie, Lake St. George and Jack's Lake, Ontario, have shown that vanadium may have both stimulatory and inhibitory effects on photosynthetic processes. Experiments with axenic cultures of algae representing three taxonomic divisions indicate more than an order of magnitude difference in response to vanadium levels. A concentration of 100 $\mu g\ \ell^{-1}$ vanadium is sufficient to kill the blue-green alga *Anabaena flos-aquae* while the green algae *Chlorella pyrenoidosa* and *Scenedesmus obliquus* and the diatom *Navicula pelliculosa* are only slightly retarded in growth at 10-100 $\mu g\ \ell^{-1}$. If this is a general phenomenon, vanadium at levels encountered as a result of industrial operations may have a profound effect on phytoplankton community structure.

INTRODUCTION

The commercial exploitation of fossil fuels of high vanadium content in recent years has led to an increased loading of the environment with this element. Both the burning and processing of these fuels have been cited as high point sources of vanadium emissions (Bengtsson and Tyler, 1976; U.S. Environmental Protection Agency, 1977).

Background concentrations of vanadium in natural waters are rather variable ranging from undetectable to 300 $\mu g\ V\ \ell^{-1}$ (Linstedt and Kruger, 1970; U.S. Environmental Protection Agency, 1977). In Ontario lakes, we have found that vanadium concentration follows the general trend of soluble metal concentrations (Lake Ontario, 6.0-6.6 $\mu g\ \ell^{-1}$; Lake St. George, 3.5-3.8 $\mu g\ \ell^{-1}$; Lake Simcoe, 6.8 $\mu g\ \ell^{-1}$; Lake Scugog, 6.1 $\mu g\ \ell^{-1}$ and Rice Lake, 4.2 $\mu g\ \ell^{-1}$). However, concentrations in industrial effluents affected by point sources may be as high as 600 $\mu g\ V\ \ell^{-1}$ (Strosher and Peake, 1976).

Very little information is available on the environmental toxicology of vanadium. Laboratory studies have indicated that the element is an essential micronutrient for certain microorganisms, such as the mold *Aspergillus niger* (Bertrand, 1941) and the alga *Scenedesmus obliquus* (Arnon and Wessel, 1953). Stimulatory effects on algal growth and metabolism in laboratory cultures have been reported. For example, 20 $\mu g\ V\ \ell^{-1}$ increased the dry weight of *Chlorella pyrenoidosa* and *Scenedesmus obliquus* by five to six fold and enhanced chlorophyll production in the former species by 83% (Meisch and Bielig, 1975).

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M1C 1A4

Fay and de Vasconcelos (1974), found that vanadate decreased algal pigmentation; inhibited nitrogenase activity and increased heterocyst formation in Anabaena cylindrica. Above a concentration of 2000 μg V ℓ^{-1}, growth inhibitory effects have been noted in several algal species (Sahay and Sankaram, 1968; Salageanu, 1973 and Meisch et al., 1977). Studies such as these indicate that vanadium may play a profound role in altering the population composition of freshwater algal concentrations.

MATERIALS AND METHODS

Field studies: Effect of vanadium on photosynthesis and extracellular release

Studies were conducted on lakewater samples from Lake Erie, Lake St. George and Jack's Lake.

Immediately after collection, the sample was distributed in 125 ml pyrex bottles and less than 1.0 ml of an aged (>1 week) vanadium stock solution (sodium orthovanadate in water adjusted to pH 7.0) was added to obtain concentrations from 10 to 5000 μg V ℓ^{-1}. ^{14}C-$NaHCO_3$ was added at the rate of 10 μCi/100 ml and the bottles were incubated in the lake at the initial sampling depths or in an incubator with continuous agitation, at ambient lakewater temperature and light intensity (quartz halogen or fluorescent floodlights).

At intervals, triplicate 10 ml aliquots were filtered using a suction of 75 mm Hg and 0.45 μ Sartorius, type SM membrane filters. The filters were promptly removed, and radioactivity was measured in a Beckman counter (Model LS-200) after addition of 15 ml of a PCS scintillation fluor (PCS; Amersham/Searle).

Extracellular organic ^{14}C substances in the filtrate were estimated as described by Nalewajko (1978); but PCS was used as the scintillation cocktail, instead of the dioxane based fluor.

The radioactivity of ^{14}C-$NaHCO_3$ added to each sample was determined by counting replicate 1.0 ml aliquots of the sample with the addition of 1.0 ml phenethylamine (Iverson et al., 1976) and 15 ml PCS.

Laboratory culture studies: Species specific response

Axenic cultures of Chlorella pyrenoidosa (Chick) UTEX26, Scenedesmus obliquus (Turp) Kruger UTEX78, Navicula pelliculosa (Breb) Hilse UTEX645 and Anabaena flos-aquae (Lyngbye) UTEX1444 were obtained from the Texas Culture Collection and maintained in Chu-10 media (Chu, 1942).

For experiments, algae were grown with constant agitation and illumination (1.75 cal m^{-2} min^{-1}), from fluorescent lights (50% "cool white": 50% "daylight" fluorescent lights). For experiments triplicate cultures were started with 1.0 ml of the appropriate inoculum (in exponential phase) and grown as above for 7 days. Cultures of Chlorella pyrenoidosa, Scendesmus obliquus and Navicula pelliculosa were incubated at 23°C, while Anabaena flos-aquae was maintained at 25°C.

Additions of vanadium to the culture media (0-1000 μg V ℓ^{-1}; as sodium orthovanadate) were made prior to adjustment of the pH to 6.8 and sterilization by autoclaving. Chemical analysis (r.f. induced plasma emission spectroscopy), of the Chu-10 media composed of analytical grade reagents indicated a background vanadium concentration of 2.7 μg V ℓ^{-1} prior to any addition.

Population density in cultures of Chlorella pyrenidosa, Scenedesmus obliquus and Navicula pelliculosa was determined using a hemocytometer. Anabaena flos-aquae was enumerated using the inverted microscopy technique (Utermöhl, 1931). Clumps of cells or filaments were disrupted by sonication prior to enumeration.

For dry weight determination, aliquots of the algae cultures were filtered onto tared pre-ashed glass-fiber filters (Whatman, GFC) and dried at 150°C to constant weight.

Chlorophyll a was extracted with dimethyl sulfoxide (Shoaf and Lium, 1976); and concentrations were calculated using the equations of Jeffrey and Humphrey (1975).

Routine tests for bacterial contamination of the cultures were carried out during the experimental period after acridine orange staining using epi-fluorescence microscopy (Lee, 1977).

RESULTS

The effects of vanadium on primary production in eutrophic Lake Erie and oligotrophic Lake St. George and Jack's Lake were investigated using the carbon-14 technique. Differences were observed between lakes (figure 1, 2a and 3) and at different depths within a lake (figure 3). Concentration of vanadium <50 μg ℓ^{-1} were, at times, stimulatory to photosynthesis, while concentrations >50 μg ℓ^{-1} consistently and significantly depressed photosynthesis (figure 1, 2a and 3). In Lake St. George, vanadium additions stimulated extracellular release of organic compounds (figure 2b).

Preliminary data on four species of algae representing three taxonomic divisions, indicated more than an order of magnitude difference in growth response (dry weight, chlorophyll a and cell number) to vanadium levels. Complete suppression of growth in the blue-green alga Anabaena flos-aquae occurred at 100 μg V ℓ^{-1}, while the green algae Chlorella pyrenoidosa and Scenedesmus obliquus and the diatom Navicula pelliculosa were only slightly retarded in growth at concentrations up to 100 μg V ℓ^{-1} (figure 4, 5, 6 and 7). Considering the suppression of cell numbers observed for Chlorella pyrenoidosa (figure 5), it is apparent that chlorophyll a content on a per cell basis is greatly stimulated by vanadium with an observed maximum effect at 10 μg ℓ^{-1}.

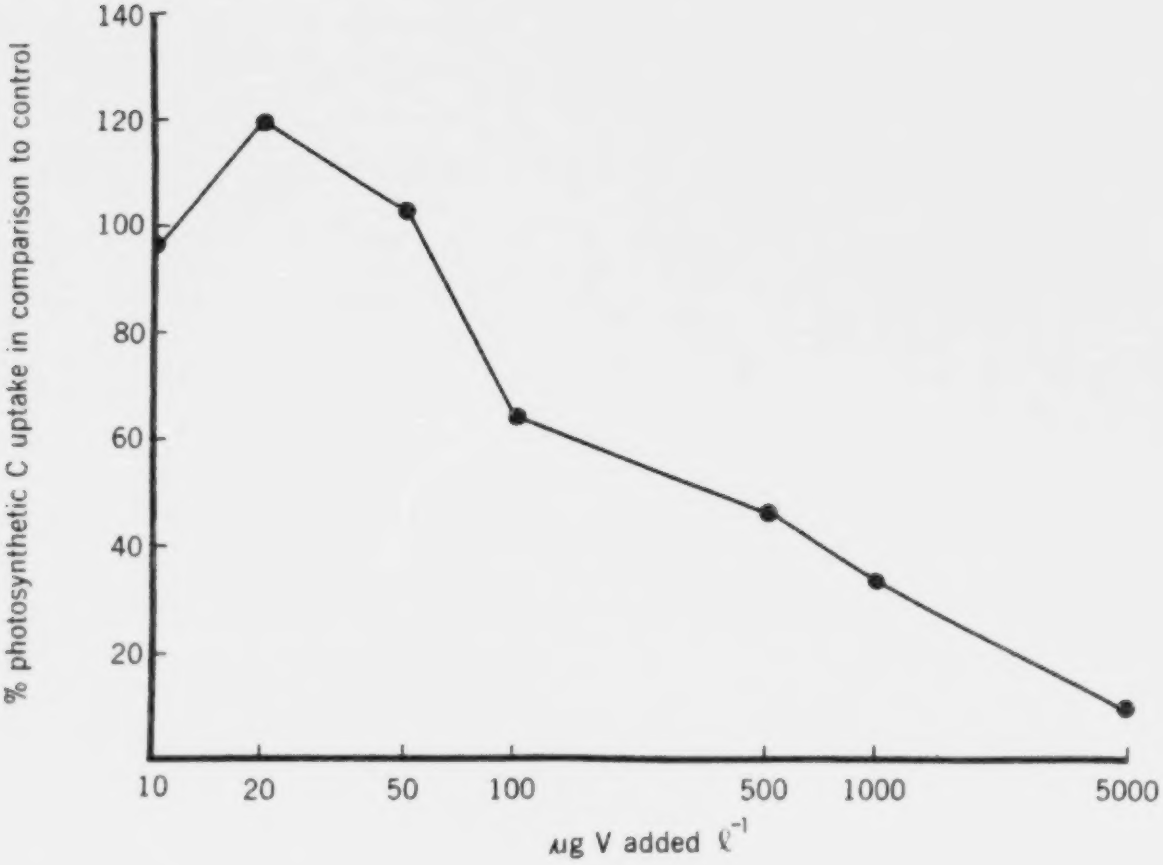


Figure 1. Effect of vanadium concentrations (0-5000 µg V ℓ^{-1}), on photosynthetic carbon uptake in Lake Erie; June 1, 1978. (Sample collected from the central basin of Lake Erie, 1 meter depth, incubated for 6 hours at ambient lakewater temperature and light conditions).

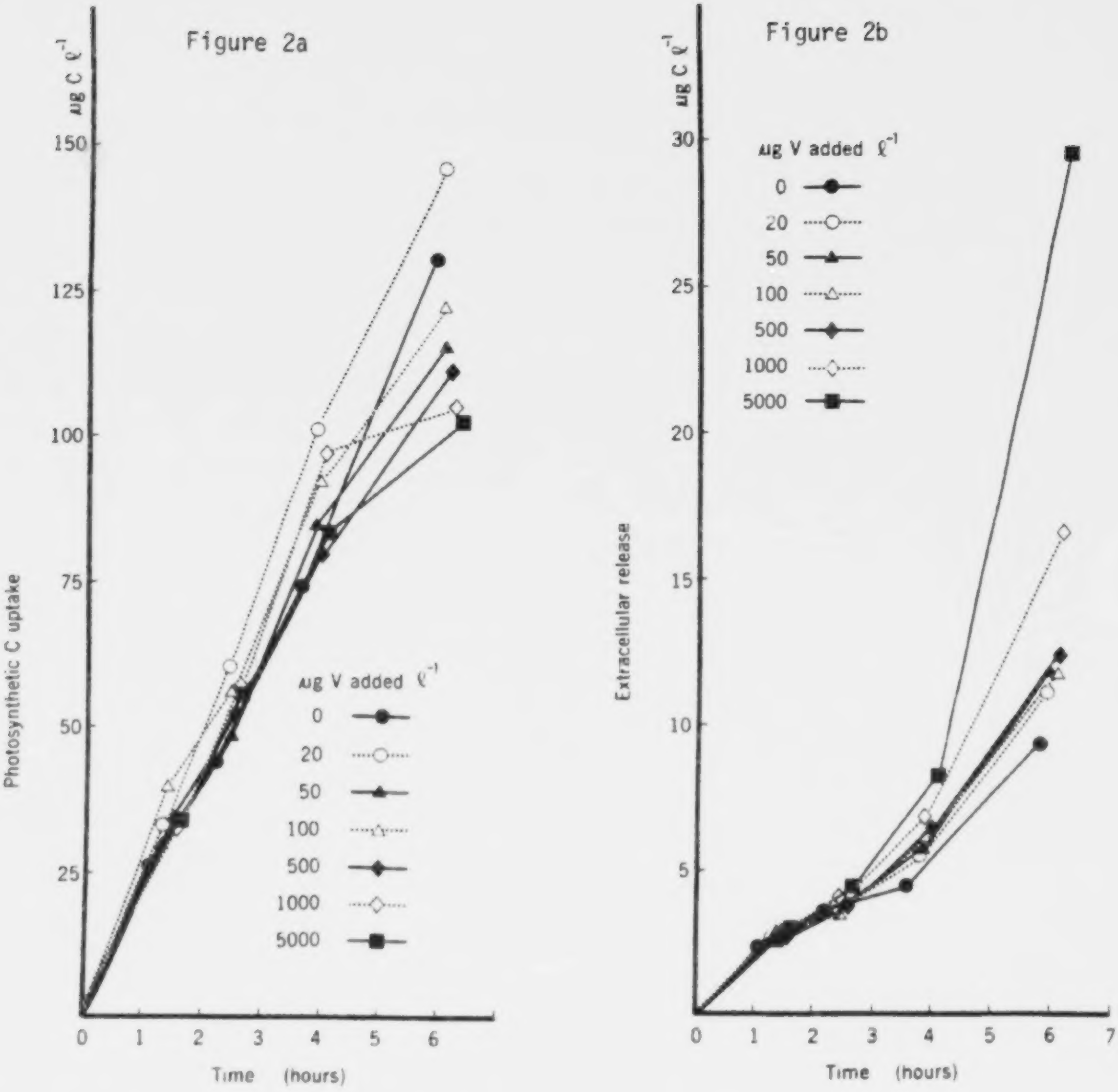


Figure 2a and 2b. Effect of vanadium (0-500 µg V ℓ^{-1}) on photosynthesis and extracellular release in Lake St. George; August 15, 1978. (Sample collected from 1 meter depth; incubated at ambient lakewater temperature and 1.75 cal. m^{-2} min^{-1} fluorescent light).

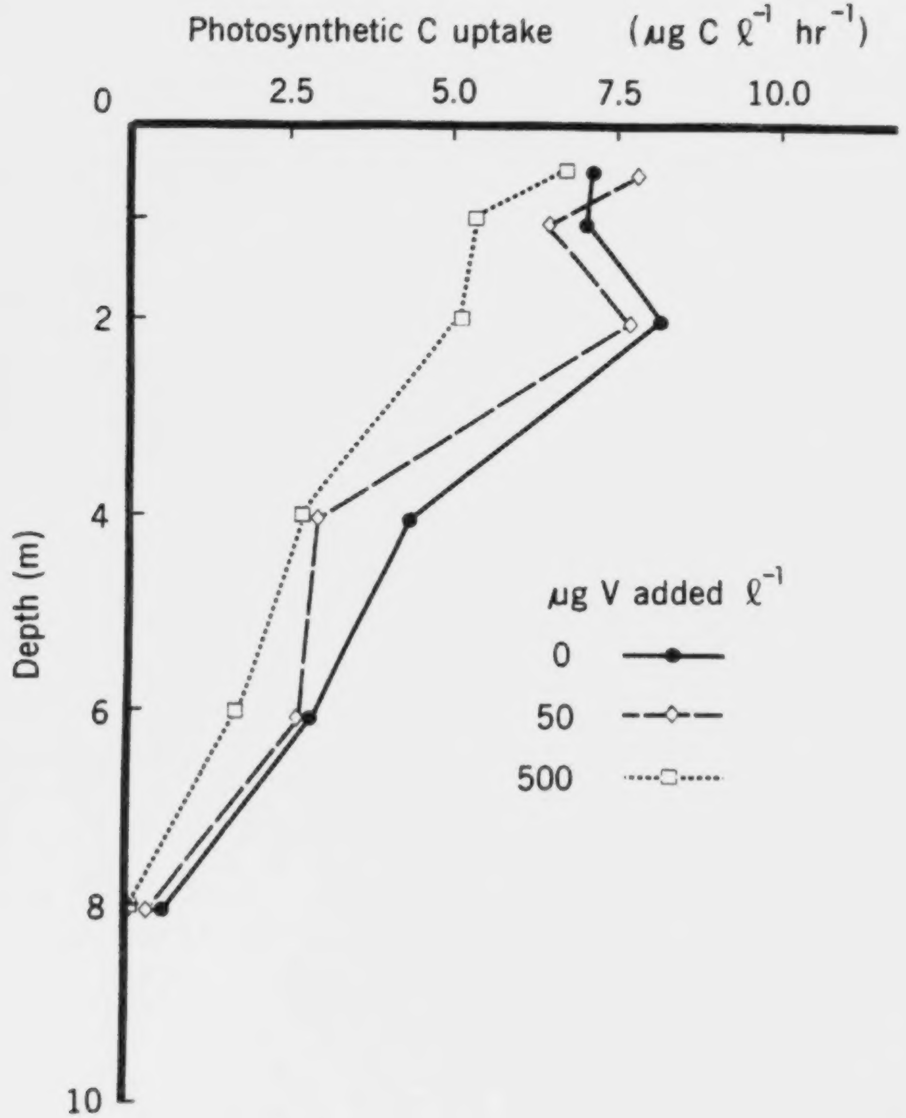


Figure 3. In situ depth profiles of photosynthesis with additions of 50 µg V ℓ-1 and 500 µg V ℓ-1 in Jack's Lake; August 23, 1978.

Figure 4

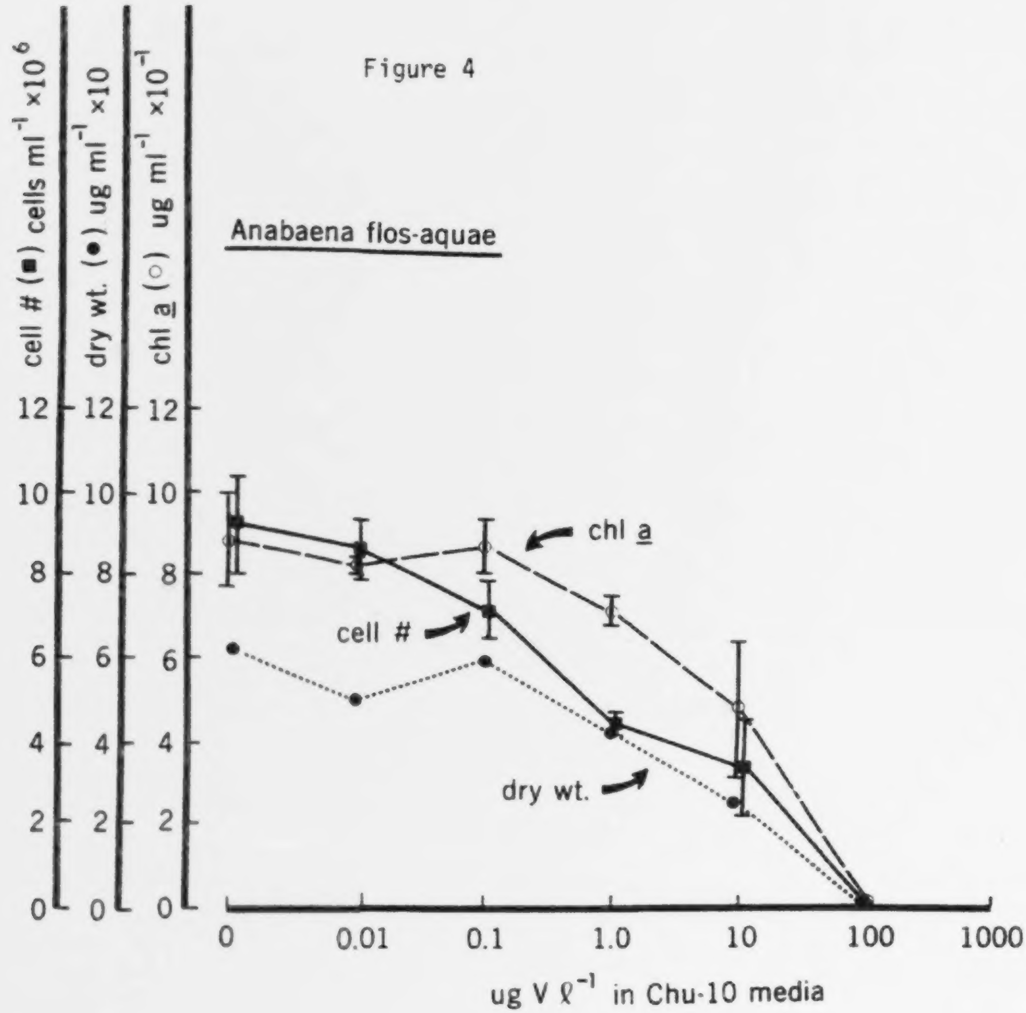


Figure 4, 5, 6, and 7. Effects of vanadium concentration on the growth of the blue-green alga Anabaena flor-aquae; the green algae Chlorella pyrenoidosa, Scenedesmus obliquus and the diatom Navicula pelliculosa.
(Data represents biomass measurements after 7 days autotrophic growth).

Figure 5

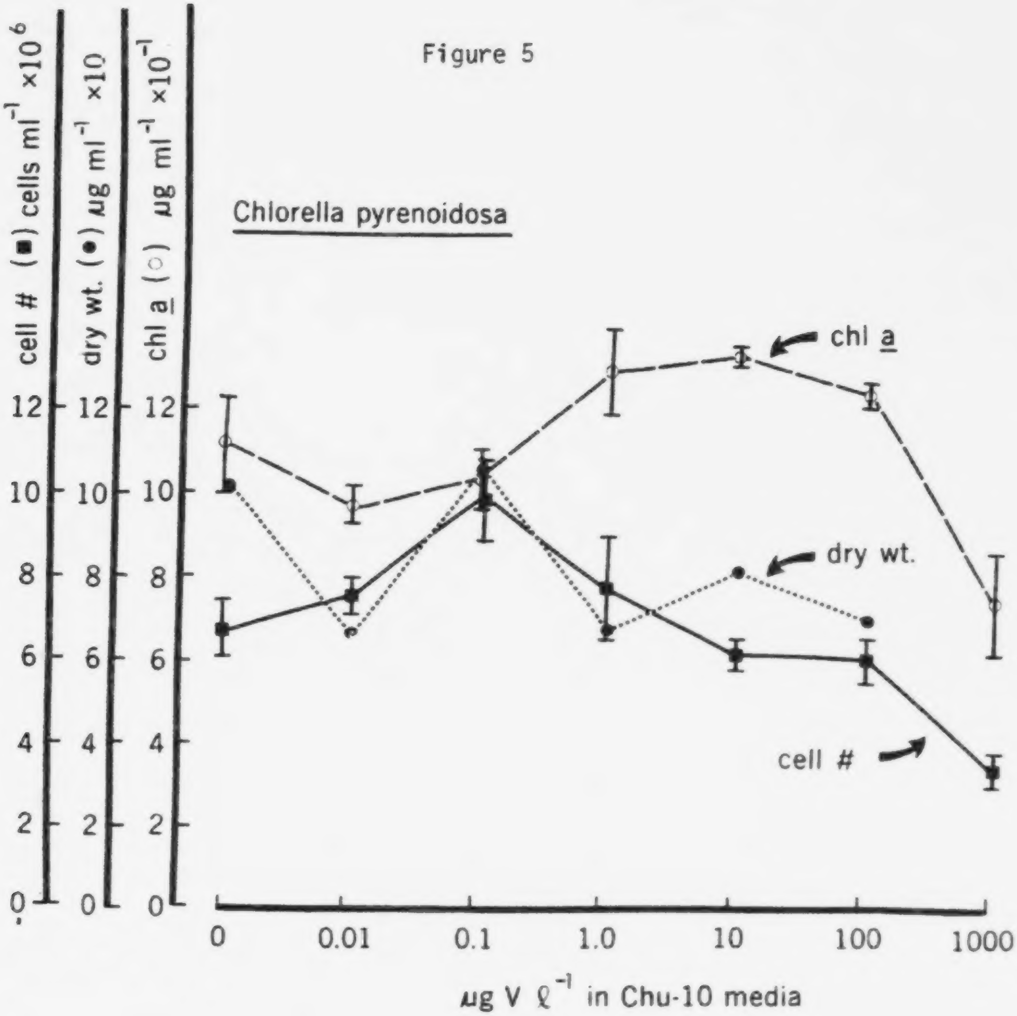

Chlorella pyrenoidosa
cell # (■) cells ml^{-1} ×10^{6}
dry wt. (●) µg ml^{-1} ×10
chl a (○) µg ml^{-1} ×10^{-1}
0
2
4
6
8
10
12
0
0.01
0.1
1.0
10
100
1000
µg V ℓ$^{-1}$ in Chu-10 media
chl a
dry wt.
cell #

Figure 6

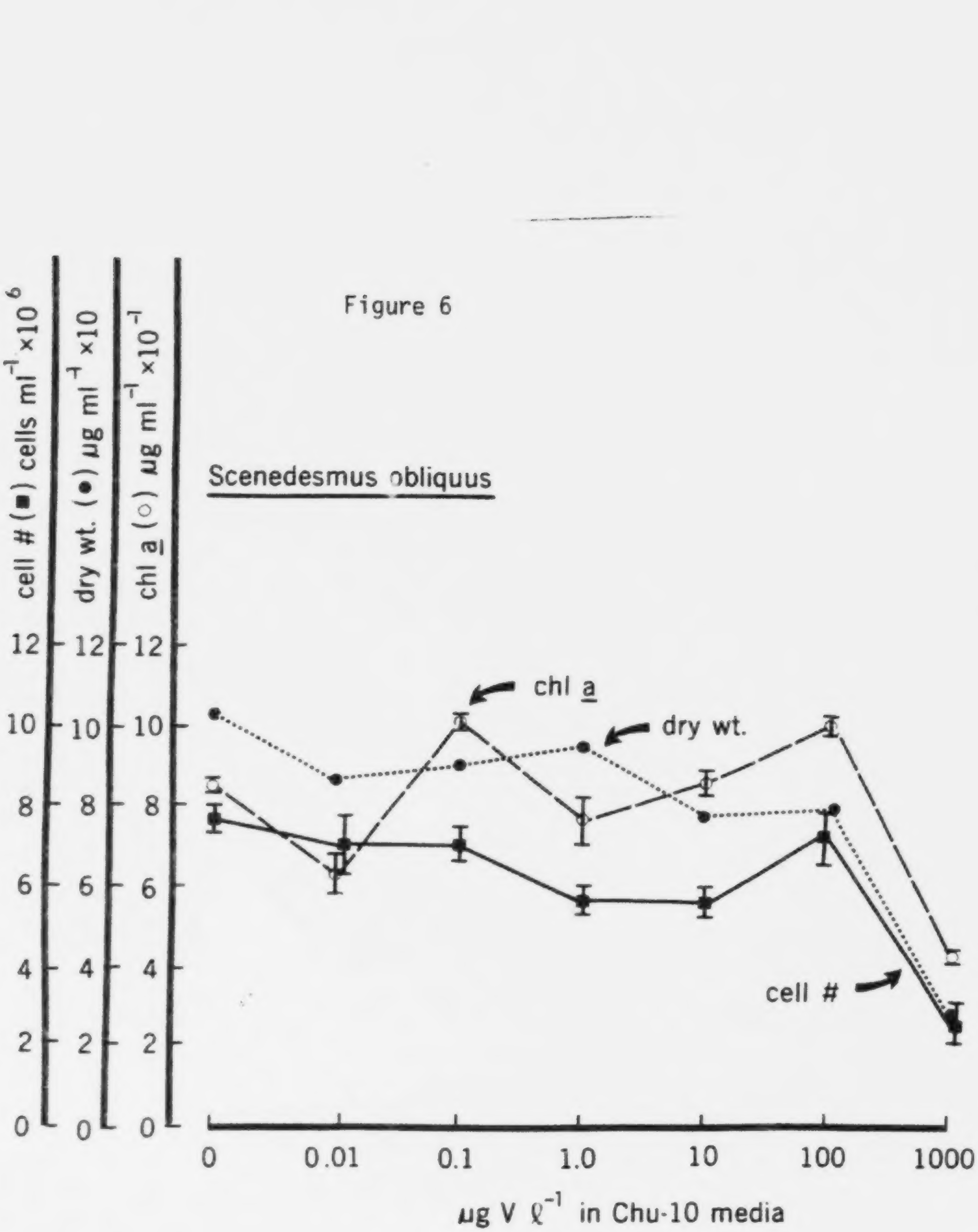
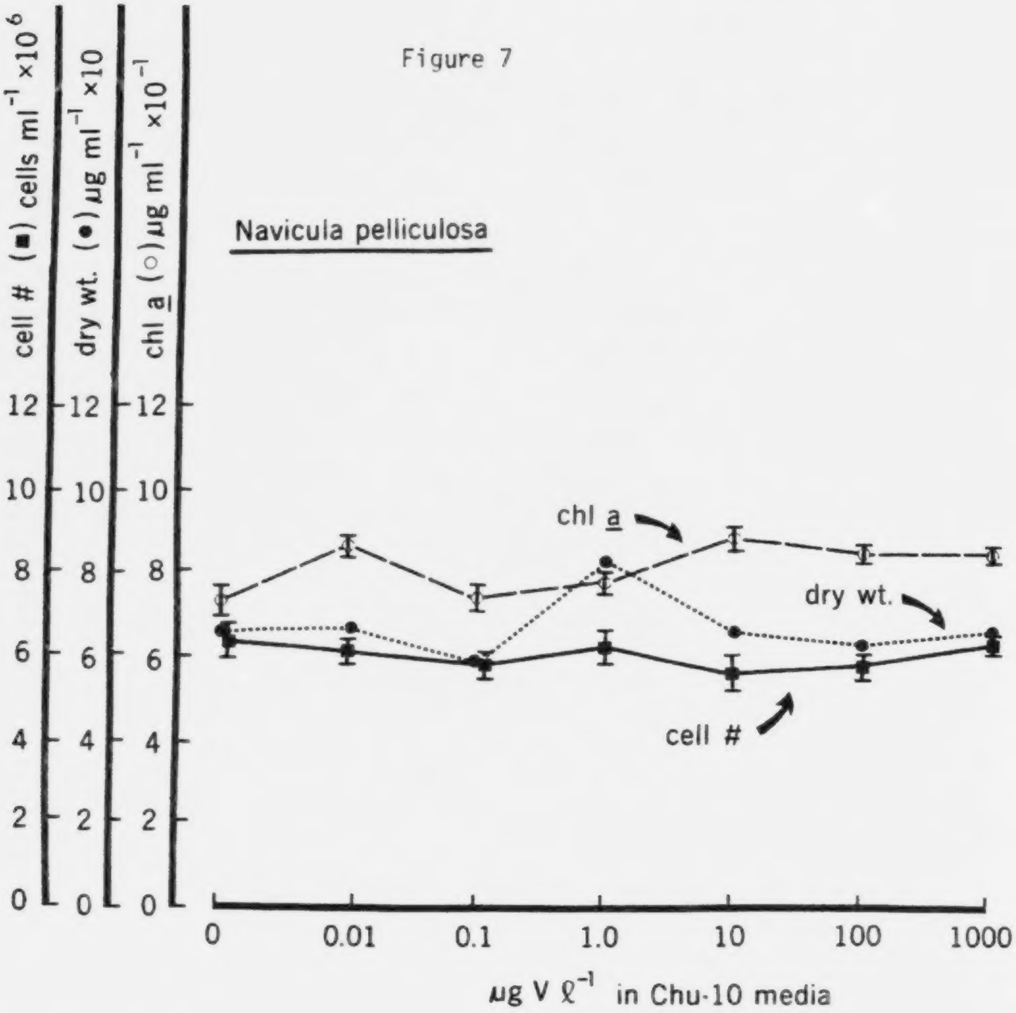

cell # (■) cells ml^{-1} ×10^{6}
dry wt. (●) µg ml^{-1} ×10
chl a (○) µg ml^{-1} ×10^{-1}
Scenedesmus obliquus
12
10
8
6
4
2
0
chl a
dry wt.
cell #
0
0.01
0.1
1.0
10
100
1000
µg V ℓ$^{-1}$ in Chu-10 media

Figure 7


cell # (■) cells ml^{-1} ×10^6
dry wt. (●) μg ml^{-1} ×10
chl a (○) μg ml^{-1} ×10^{-1}
Navicula pelliculosa
chl a
dry wt.
cell #
0
2
4
6
8
10
12
0
0.01
0.1
1.0
10
100
1000
μg V ℓ$^{-1}$ in Chu-10 media

DISCUSSION AND CONCLUSIONS

Stimulation of photosynthesis of natural populations (figure 1, 2a and 3) by low concentrations of vanadium (<50 μg ℓ^{-1}) is consistent with previous research reports. Arnon (1958), first noted that photosynthetic rate in laboratory cultures of Scenedesmus obliquus could be increased two fold in the presence of vanadium under high light (20,000 lux) conditions. Under lower light (2,000 lux) intensities, no significant stimulation was observed. Chlorophyll production in Scenedemus obliquus can also be stimulated in the presence of vanadium (Meisch and Bielig, 1975). The stimulatory effect noted in our field studies could arise from either a rate increase in photosynthesis or from enhanced pigment production.

Suppression of photosynthetic rate was observed in natural lakewater samples with concentrations of added vanadium >50 μg ℓ^{-1} (figure 1, 2a, and 3). The difference in sensitivity of the algal communities in the different lakes or at different depths in a single lake, may reflect species specific responses or be related to differences in environmental factors such as light or nutrients. High concentrations of vanadium have been previously reported to be toxic to algae (Sahay and Sankaram, 1968) and to suppress vital metabolic functions such as nitrogen fixation in the blue-green algae (Fay and de Vasconcelos, 1974).

The addition of vanadium causes an increase in extracellular production of organic compounds by the algae as shown (figure 2b). These excreted organic compounds may act as chelating agents which complex the vanadium and diminish the concentration of free vanadate. This process may influence the concentration dependence of vanadium induced effects.

Relatively high background vanadium concentrations in Chu-10 mediums (2.7 μg V ℓ^{-1}) could mask responses to low additions of vanadium. For example, the continuous increase in the dry weight of Chlorella pyrenoidosa at added concentration of 0.01 to 1.0 μg V ℓ^{-1} as reported by Meisch et al., 1977. Studies on Chlorella pyrenoidosa have shown maximum stimulation of dry weight and chlorophyll content at 500 μg V ℓ^{-1}; suppression of growth at concentrations greater than 25 mg V ℓ^{-1} and a lethal concentration of 100 mg V ℓ^{-1} (Meisch et al., 1977). Our results indicate complete suppression of growth of Anabaena flos-aquae at 100 μg V ℓ^{-1} and minor suppression of growth of Chlorella pyrenoidosa and Scenedesmus obliquus at concentrations of 1 mg V ℓ^{-1}. No significant effect on Navicula pelliculosa at concentrations as high as 1 mg V ℓ^{-1} has been observed. The lack of an understanding of vanadium toxicity mechanisms in algae makes meaningful comparisons of data from various sources difficult at this time. Difference in growth conditions in the various studies may alter the sensitivity of organisms toward vanadium. Patrick et al. (1975); and Meisch et al. (1977); have suggested that the intracellular concentration of vanadium is the determining factor in cell response and that the concentration of vanadium in the medium may not be pertinent. Thus Patrick et al., (1975), further suggests that the build up of intracellular concentration may depend upon environmental factors controlling the rate of cell division.

The causes of algal growth inhibition by vanadium have not been elucidated. Lepp (1977), suggests that vanadium toxicity in plants may be due to enhancement of enzyme activity, particularly those involved in regulating hormone levels. Tyler (1976) has shown that vanadium can significantly reduce the acid phosphatase activity in soils.

In our laboratory studies, the diatom *Navicula pelliculosa* showed remarkable tolerance to vanadium concentrations up to 1 mg ℓ^{-1}; while the blue-green alga *Anabaena flos-aquae* was found to be highly sensitive to vanadium concentrations as low as 100 μg V ℓ^{-1} (figures 7 and 4). Studies on the response of diatom communities on slides (principally *Melosira* and *Synedra*), to trace elements conducted by Patrick et al., 1975, showed a shift from diatom dominant communities to blue-green algae dominated communities, (species not identified), at vanadium concentrations >4 mg ℓ^{-1}. Concentrations up to 20 μg V ℓ^{-1} appeared to favour diatom growth over other algal species present.

Our results and those of Patrick et al. (1975); indicate that vanadium levels such as those encountered as a result of industrial operations, may have important effects on the population structure of aquatic ecosystems in the receiving waters. Further quantitative documentation of the effects of vanadium on algal communities as a basis for the incisive investigation of the mechanism involved in these effects is currently in progress.

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APPENDIX I

DISCUSSION WORKSHOP REPORT

WORKSHOP 1 - NEW TECHNIQUES IN TOXICOLOGY. CHAIRMAN: G. CRAIG.

1. Locomotor activity testing using sonar beam interruptions by E. Sherer and S. Harrison.

The 1977 Workshop in Vancouver focused on *in situ* evaluations of effluent discharges on aquatic organisms and this paper provided yet another technique that might be used to measure fish responses in the field. Although the technique itself is still under development a number of refinements have been incorporated to improve the response reliability.

The advantages of the system include the use of large groups of fish, the equipment is reasonably portable allowing transportation to isolated areas and relocation within certain areas of the site to provide base line data. Mixing zones might be identified, up and downstream comparisons may be completed and the entire system is battery operated.

Some biological interferences remain to be identified such as general interaction among fish and the increased activity of fish during feeding. The ratio of effective concentrations producing behavioural change to lethal concentrations has yet to be established. The potential problem of fish accommodation to initial effective concentrations has also not been investigated.

The activity data is collected on paper tape, transported to the lab for analysis by computer, which simplifies the statistical evaluation but does require appropriate computer support.

This type of monitoring shares with other cage exposure systems a high susceptability to vandalism which may either limit its applicability or increase the cost of operation by requiring additional surveillance. Manufacture of the electrical components, which are not commercially available, makes construction expensive for independent laboratories and requires a highly skilled electronics person to assemble and maintain the various modules.

Although designed for field use this fish activity monitor may be best suited for the laboratory or some other permanent installation where technical services are readily available and staff support can be minimized.

2. A recycle holding system for freshwater and marine fish stocks by A.L. Beckett and B.T. Thackeray.

Where available water quality or quantity limits the operation of a bioassay laboratory recirculation of holding water may solve the inherent problems of water usage.

The most expensive component of the system is the compressor and estimating the energy requirements is critical to successful operation. A good knowledge of incoming water temperature during summer months and room temperature control is also important to size the chiller unit.

The holding tanks, plumbing, pumps and other equipment are all commercially available and the water treatment system is reasonably inexpensive and easy to construct which makes the unit attractive.

The treatment chamber filter must be inoculated and started slowly, gradually increasing the fish load until full capacity can be maintained. Ammonia levels of 10 mg l^{-1} can be effectively reduced to non-detectable after a single pass through treatment chamber. Unfortunately the biological system will not withstand any treatment of fish stocks with antibacterial agents that might be used to remedy disease problems.

3. Three bioassay techniques for demonstrating toxicity to aquatic biota by C.I. Mayfield, W.E. Inniss and J.E. Thompson.

Hazard assessment techniques are increasing in scope among bioassay laboratories where multiorganism evaluations are being favoured to determine the effects of toxicants on several trophic levels of aquatic biota. The most appealing techniques in this presentation were those dealing with changes in membrane phase transition temperature characteristics and fluorescent staining of micro organisms to monitor growth rates in sediments and water.

Although algae from field collections and pure cultures provided the membrane material for the phase transition temperature evaluation, it was suggested that this technique could be applied to any biological membrane system. Changes in these membrane characteristics result in alterations in structure and potential function. Responses are non-specific with respect to toxicants and would not provide compound identification. It was also recognized that biological interferences such as aging could also alter membrane phase transition temperature characteristics.

Fluorescent staining of micro-organisms allowed _in situ_ monitoring of growth rates in sediments and water thus providing an indication of toxic responses to single compounds or mixtures.

Application of these techniques could be used in assessing the biological activity of complex compounds or wastes. The fluorescent staining technique appears to be reasonably applicable in routine bacteriological or histological laboratories while the membrane phase transition temperature methods are technically more demanding, time consuming and expensive.

WORKSHOP 2 - BIOCHEMICAL METHODS FOR TOXICITY EVALUATION (ATTENDANCE ABOUT 30).
CHAIRMAN: J. KLAVERKAMP

The intent of this workshop was to discuss the applicability of using specific enzymatic and other biochemical responses of individual organisms to toxic substances control. Both papers presented in this session provided an excellent starting point. The contribution by Ray Morgan (follows) reviewed the "State of the Art". Dr. Morgan demonstrated what information could be derived from this approach plus the characteristics of the ideal method, i.e. that it be sensitive, specific, sensible, that baseline norms be established, etc. He indicated that a rather extensive review of the literature identified few common threads and that much of the work today was piecemeal, misapplied or irrelevant. However, he saw a high potential for future applications. During the following discussion, P. Hodson identified three useful types of information to be derived from studies of toxicant effects on enzymes. The first with the effects on specific enzyme characteristics. Studies of the response of a specific enzyme itself would provide valuable information on the molecular mode of action and on structure activity correlations. Secondly, responses of specific enzymes to toxicants could be related to specific human or fish health problems, i.e. the induction of enzymes by precarcinogens can be related to turnover development. Thirdly, enzyme responses often provide a direct indicator of the degree exposure and overall response of an intoxicated organism. This was particularly valuable in aquatic toxicology where "dose" is unknown and contaminant residue analysis of small tissue samples may be impossible or too expensive.

The second paper by R.F. Addison described a specific enzyme system - The Mixed Function Oxidase system (follows). The value of this paper was its demonstration of the utility of enzymes as indicators of exposure and of the variation between phyla of response, i.e. chemicals inducing MFO enzymes in rats did not necessarily do the same thing in fish. Recognition of these factors obviously would increase the activity of this system.

Dr. W. Penrose contributed a paper that was not listed on the program. It demonstrated the utility of the liver aryl dehydrogenase and aryl hydroxylaseplus liver, spleen and gonadal somatic index as indicator in fish of exposure to oil. This method, applied to local resident fish, has the potential as a good indicator of low level chronic oil pollution.

Further discussion raised some more good points. Dr. G. Leduc pointed out that not all responses to toxic materials are adverse, that these responses may represent accommodation or a beneficial supra normal response at a low level of stress. One example given was that cyanide has a depressant effect on fish metabolism which may allow the fish to survive other adverse conditions.

Dr. P. Anderson raised the question of contaminants in control fish diets. He suggested that increases or decreases in activity of enzymes, representative of induction or toxicity, may be masked by responses to pre-exposure to contaminants in the control diet.

Other suggestions raised during discussion included a consideration of biota other than fish and research on tissues.and enzymes unique to each species. Overall the papers were very well presented, discussion was lively and many important issues were aired.

WORKSHOP 3 - INVERTEBRATE TOXICOLOGY. CHAIRMAN: B. WILSON

Discussion of priorities for future aquatic toxicology workshops at the 1977 meeting in Vancouver indicated a clear consensus of opinion on the subject of workshop topics. It was felt that discussion workshops should focus on narrow, easily defined topic areas, with small discussion groups tackling discrete problems. Since at least 95% of all animal species are invertebrates, the workshop entitled "Invertebrate Toxicology" at the 1978 meeting in Hamilton fell somewhat short of meeting the criteria set down the previous year. In spite of the breadth of the topic, however, and the fact that only four formal papers were presented, the area was represented well in a number of respects. Environments ranged from salt water through brackish water to fresh water, both benthic and pelagic habitats were considered, and toxicants included heavy metals, PCB's, and pesticides. Although species interest was centred on the Arthropod and Annelid phyla, this restriction does reflect the bias in the general literature.

The papers presented were well received and generated considerable discussion, with a number of problem areas attracting particular interest. Some concern was expressed about the difficulties of measuring and comparing uptake of toxicants in small invertebrates considering the great diversity in methods of feeding, respiration, exoskeleton formation and composition, etc. Considerable time was also given to considerations of heavy metal interference in the processes involved in crustacean moulting. It was felt that the physiology of ecdysis should be a promising area for research in sublethal toxic effects. As well, there was some interest in the role of the exoskeleton as a deposition site for the packaging and handling of toxicants.

It was felt that future discussion workshops in this topic area might better be served by more restricted subjects (possibly determined by a pre-workshop poll of potential contributors) and by fewer formal papers (preferably one review paper and one current research paper), with more time for discussion and informal presentations.

APPENDIX II

Aquatic Toxicity Research in Canada

P.V. Hodson

Current restrictions on spending have increased the necessity to avoid duplication of effort. To provide a fairly fast, timely review of aquatic toxicity research in Canada, surveys were solicited from individuals in the various regions and the results were presented at the workshop. The following table is a summary of what was presented and the editors apologize in advance to those whose research have been overlooked, misrepresented or oversimplified. A longer, more detailed presentation would take so long to publish as to be totally out of date. Sincere thanks is extended to the following people who complied this information.

Atlantic region - P.G. Wells
Quebec - G. Leduc
Ontario - G. Craig, P.V. Hodson
Western region - J. Klaverkamp, B.T. Thackeray
Pacific region - R. Hoos

Location	Person or Group	Material	Parameters
A NEWFOUNDLAND			
I Fisheries and Environment Canada			
a) FMS Biological Station, St. John's	W. Penrose	petroleum, hydrocarbons	fish - contaminant metabolism as indicated by liver aryl hydrocarbon hydroxylase
	A. Walton, W. Penrose J. Kiceniuk, D. Idler J. Green	petroleum hydrocarbons	*Tautogolabrus* (cunner) - chronic toxicity, reproduction, hormone levels, bioenergetics
	J. Payne	mutagens	Ames test - sea water, crank-case oil, rotting fish
	J. Kiceniuk	oil spill dispersants	cunners - cardiovascular function
	J. Payne, J. Kiceniuk G. Fletcher	oils	flounders, cunners - chloride levels
b) EPS Service Bioassay Lab., St. John's		industrial effluents	toxicity monitoring
II Memorial University, St. John's			
a) Biochemistry Dept.	P. O'Brien	mutagens	aquatic environment - fate and accumulation
b) Biology Dept.	R. Thompson	petroleum	scallops - toxicity

Location	Person or Group	Material	Parameters
B NOVA SCOTIA			
I Fisheries and Environment Canada			
a) FMS Halifax Laboratory	J. Uthe, H. Freeman M. Li, G. Sirota	PCB's	cod - endocrinology, histopathology - reproductive and metabolic functions
			cod, herring, salmon, lobster - enzymology of reproduction and egg development
		Persistent Contaminants	cod - liver and muscle residue monitoring mussels - "mussel watch" monitoring
		hexachlorobenzene	cod - effects on liver function
b) Bedford Institute of Oceanography			
(i) Marine Ecology Labs.	R.F. Addison	test development - halogenated and aromatic hydrocarbons	brook trout - mixed function oxidase induction
	D. Loring	heavy metals	fractionation in sediments and availability to biota
	G. Harding	DDT, PCB's	copepods - uptake from diet, dynamics, modelling
	J. Vandermeulan	test development	Monochrysis lutheri (dinoflagellate) - behaviour when exposed to pollutants

Location	Person or Group	Material	Parameters
(ii) Resource Development Branch	G. Farmer	acidity	Atlantic salmon - mortality of fry -
		anti-fouling paints	Atlantic salmon - toxicity
(iii) Environmental Protection Service	G. Westlake	Kraft Mill Effluents	*Daphnia*, rainbow trout - toxicity
	P. Wells	test development - formaldehyde	green sea urchin - behaviour
	P. Eaton	chlorobenzenes, metals, pesticides, petroleum hydrocarbons, chlorinated hydrocarbons	sediments, biota - contamination
II Nova Scotia Research Foundation, Dartmouth	K. Hellebrand	industrial effluents	routine toxicity testing
		Kraft Mill Effluent	*Spartina* (Marsh grass) - Nitrogen fixation
III McLaren-Marex Ltd., Dartmouth	M. Hutcheson	cadmium	partitioning in geochemical compartments - biological accumulation from each compartment
		dispersant - oil mixture	Arctic benthos - toxicity
C NEW BRUNSWICK			
I Fisheries and Environment Canada			
a) St. Andrew's Biological Station, St. Andrew's, New Brunswick	V. Zitko	pyrethroids	fish - acute toxicity
		brominated benzenes	review

Location	Person or Group	Material	Parameters
		organohalogen contaminants	bluefin tuna, salmon - residues
	D. McLeese, S. Ray	copper, zinc, cadmium, thallium	fish, lobsters, clams, sand shrimp - toxicity, accumulation temperature effects
		organophosphate insecticides	lobsters - toxicity clams, mussels - uptake
		chlorinated hydrocarbons	*Crangon* (sand shrimp) - toxicity in sea water and sediment
		PCB's cadmium	*Nereis, Crangon* - uptake from sediment
II University of New Brunswick, St. John	M. Thomas	oil	benthos - recovery from exposure in intertidal zone
D QUEBEC			
I Fisheries and Environment Canada, EPS, Capitaine Bernier Lab, Longueil	N. Bermingham	effluents	fish - toxicity monitoring
II Service Protection de l'Environment de Quebec, Quebec City	M. Joubert	effluents	fish - starting a toxicity monitoring program
III Domtar Pulp and Paper, Montreal		effluents	fish - toxicity
IV Noranda Research Centre, Pointe Claire	M. Speyer	effluents	fish - toxicity

Location	Person or Group	Material	Parameters
V Pulp and Paper Research Institute of Can. Pointe Claire	Dr. Wong, T.G. Kovacs	effluents	fish - toxicity
IV Eco-Research Pointe Claire	C. Dumouchel	effluents	fish - toxicity
VII Concordia University, Montreal			
a) Sir George Williams Campus,	S.M. Ruby	methoxychlor, cyanide	fish - reproduction
(i) Dept. of Biology	P.D. Anderson	heavy metals	fish - multiple toxicity
	E. Malley	heavy metals	fish, *Daphnia* - chronic toxicity
	G. Leduc	methoxychlor, cyanide	fish - chronic toxicity
	S. D'Apollonia	toxicants	fish - biochemical responses
(ii) Dept. of Chemistry	J. Dick	organics and inorganics	residues - analytical chemistry
VIII Laval University, Quebec City Dept. of Biology, Chemistry, and Geology		organics and inorganics	residues - analysis
IX McGill University MacDonald College Ste. Anne de Bellevue	A. MacKenzie	pesticides	water, soil - contamination
X University of Quebec, Quebec City		mercury, lead	Freshwater and marine ecosystems - inventories and distribution in Quebec

Location	Person or Group	Material	Parameters
XI Ecole Polytechnique Université de Montréal, Montreal	C. Delisle	mercury, acidity	freshwater ecosystem - distribution
E ONTARIO			
I Fisheries and Environment Canada			
a) Great Lakes Biolimnology Lab., CCIW, Burlington	P.T.S. Wong, Y.K. Chau	Pb, As, Se, metal mixtures chlorinated benzenes	algae - toxicity bacteria - methylation
	U. Borgmann	Pb, Cd, As, Se, Chlorinated benzenes	zooplankton, snails, crayfish - growth, reproduction and effects on electron transport system
	P.V. Hodson, J. Hilton	Pb, As, Se, pentachlorophenol chlorinated benzenes	fish - chronic toxicity, factors affecting chronic toxicity, post-exposure stress, chromosome breakage as an indication of carcinogens, dietary interactions with toxicity, biochemical indicators experimental ecosystems - contaminant dynamics
	S. Millard	PCB's, Hg, Chlorinated benzenes	experimental lake ecosystems-effects and contaminant dynamics
	A. Niimi	PCB's, hexachlorobenzene	fish - contaminant dynamics and energetics

Location	Person or Group	Material	Parameters
b) EPS CCIW, Burlington	S. Metikosh	industrial effluents, pentachlorophenol	fish, Daphnia magna - acute toxicity in relation to treatment procedures, chronic toxicity
c) EMS CCIW, Burlington	K. Kwasniewska D. Liu	chlorinated organics	bacteria - effects on growth
d) CWS Ottawa	D. Hallett	chlorinated organics polynuclear aromatic hydrocarbons	clams - residues - indicator of local contamination
II Ontario Ministry of the Environment, Rexdale	G.R. Craig, C. Inniss	industrial organics - styrenes, aromatics, naphthalenes	fish - toxicity, uptake, depuration
		organics, metals	fish - activity - continuous monitor of water quality
	D. Wells	industrial organics	fish - taste impairment
	J. Reinke	industrial organics	fish - waste treatment effects on toxicity
	K. Suns, C. Curry	test development - pesticides, organics, metals	fish, clams - contaminant monitoring system, *in situ* bioassays
		mercury, acidity	fish - uptake as a result of pH interactions
	K. Holtze, H. Clarke K. Flood, T. Lagan	test development	fish fry, *Daphnia* - more sensitive toxicity tests
	J. Munro, K. Flood	steel industry effluents	fish - toxicity

	Location	Person or Group	Material	Parameters
III	University of Waterloo, Waterloo, Ontario	C. Mayfield	heavy metals, pesticides	toxicity to benthic bacteria, membranes as indicator of toxicity, chemostats
		J. Dodson	pesticides, herbicides	fish - behaviour
IV	McMaster University, Hamilton, Ontario	R. Sonstegard	chlorinated organics	fish - environmental carcinogenesis, goiterogenesis
		C. Wood	acidity	fish - pH regulation - effects of acidity
		G. Harris	nutrients, heavy metals	field ecology
V	University of Western, Ontario, London, Ontario	D. Ogilvie	acidity	fish - temperature selection
VI	Laurentian University, Sudbury, Ontario	J.R. Morris	heavy metals	algae - toxicity
VII	University of Ottawa, Ottawa, Ontario	F. Brillon	heavy metals	algae - uptake as a function of speciation
		B. Philogen	pesticides	aquatic insects
		Q. LaHam	cadmium	fish
		S. Qadri	pesticides, mirex	fish, invertebrates - uptake
VIII	University of Guelph, Guelph, Ontario a) Dept. of Zoology	G. Dixon, W. Bannis J. Sprague	copper and other toxicants	fish - acclimation effects on toxicity
		D. Stendahl, J. Sprague	vanadium	fish - pH and hardness effects on toxicity

Location	Person or Group	Material	Parameters
	R. Bradley, J. Sprague	zinc	fish - pH and hardness effects on toxicity, sublethal effects
	J. Leatherland R. Sonstegard	organochloride pesticides, Mirex PCB's	fish - effects on thyroid hyperplasia, effects of dietary administration
	B. Wilson, J. Roff U. Borgmann	lead, cadmium	zooplankton - factors affecting acute toxicity, effects on phototaxis
	C. Wren, H. McCrimmon	mercury	fish, invertebrates - effects of pH and alkalinity on uptake
	D. Gaskin	mercury, organochlorine pesticides	mammals, birds - residues in biota from Bay of Fundy
	R. Frank, R. Stewart K. Ronald	pesticides, heavy metals	harp and hood seals - residues
	D. Rodgers, F.W. Beamish	mercury	fish - uptake from water and food
	A. Kamaraguru F.W.H. Beamish	mercury, pesticides	fish - effects on energetics
	N. Weinstein F.W.H. Beamish	heavy metals	fish - RNA as a growth indicator in studies of metal mixtures
b) Environmental Biology	F. McKuen and co-workers	pesticides	ecosystems - integrated study of effects on ecosystem interactions

Location	Person or Group	Material	Parameters
IX York University Toronto			
a) Biology Dept.	M. Boyer	organophosphate pesticides	zooplankton, phytoplankton - population dynamics in small ponds
	B. Coleman N. Bermingham	organophosphate pesticides (abate, Dursban)	algae - growth, photosynthesis, nitrogen fixation
X Lakehead University	G.W. Ozburn, D. Ori	PCB substitutes	fish - chronic toxicity, reproduction, uptake from diet and water
XI University of Toronto Toronto			
a) Dept. of Preventive Medicine	A. Skobola	copper	fish - toxicity, accumulation, organ distribution, dose-response relationships
b) Botany	P. Stokes, G. Mierle	nickel, copper	algae - toxicity, synergism, membrane leakage
	J. Hellebust, T. Hutchinson	napthalene, benzene, toluene, xylene, aqueous extracts of crude oil	algae - toxicity related to partition coefficients
	T. Hutchinson	arsenic, copper, cadmium, nickel, zinc, acidity	phytoplankton, zooplankton, benthos - growth, photosynthesis, membrane ion permeability

Location	Person or Group	Material	Parameters
		aluminum, manganese, zinc, acidity	zooplankton, chironomids - Na^+ - K^+ regulation in Tundra lakes undergoing acidification
	T. Hutchinson, T. Hall	nickel	zooplankton - uptake, depuration
c) Institute for Environmental Studies	A. Forrester	heavy metals	clams, aquatic invertebrates - uptake dynamics from sediment
d) Dept. of Zoology	G.F. Holeton	chlorine	fish - physiology
	C. Neville	acidity	rainbow trout - respiration, acid base balance, electrolyte balance
	H. Harvey	acid precipitation, metals	fish - toxicity; ecosystem approach to predictive models
e) Erindale College	A. Zimmerman J. Paloheimo	pH, metals	ecosystems - response, computer modelling
f) Scarborough College, West Hill	C. Nalewajko	vanadium	algae - toxicity, photosynthesis, growth
	A. Witherby, S. Rogers	test development, zinc	fish - monitoring physiological responses of free-swimming fish
	D. Williams	stream substrate manipulation	aquatic insects - environmental impact assessment
XII McLaren Consulting, Toronto	T. Watson	mercury, acidity	fish - model ecosystems - acidity uptake of mercury

Location	Person or Group	Material	Parameters
F MANITOBA			
I Fisheries and Environment Canada, Freshwater Institute, Winnipeg	B.G.E. de March	test development	*Hyallela azteca* (amphipod) - reproduction, growth impairment
	R.E. Evans, J.J. Hara	test development	fish - localization of phospholipids in olfactory epithelium as an indicator of toxicity
	J.F. Flannagan D.G. Cobb	test development with trisodium NTA	*Helisoma trivolvis* (snail) - growth, reproduction, mortality
	M.K. Friesen	test development	*Hexagenia rigida* (mayfly) - embryonic and hatching characteristics of eggs
	M.A. Giles, D. Klaprat	test development, whole effluents, heavy metals	fish - residual oxygen bioassay
	T.J. Hara	test development - toxicants, environmental change	fish - response to toxicants of olfactory bulbar electrical response to standard stimuli
	F.P. Healey	test development	algae - growth rate in batch cultures
	H.S. Majewski, J.F. Klaverkamp	test development - organophosphate insecticides, organic solvents, heavy metals	fish - cardiovascular and respiratory function
	D.F. Malley	test development - acidification, heavy metals	*Orconectes virilis* (crayfish) - uptake of ^{45}Ca after moulting

Location	Person or Group	Material	Parameters
	E. Scherer, S. Harrison	test development	fish, invertebrates - behaviour, avoidance, locomotor activity, response to overhead light, optomotor response
	B. Townsend	test development	*Chironomus tentans* (insect) - multigeneration studies
	T.J. Hara	test development - organophosphate and carbamate insecticides, copper, cadmium, mercury	fish - binding/uptake kinetics on chemosensory membranes as it affects stimulus - response - uptake of neurotransmitter by neurons (ACh and AChE) - behaviour - reactions to biological stimulants - mucous secretion
	B. Evans	test development - organophosphate insecticides, cadmium	fish - histopathology, histochemistry - localization of AChE in brain - light and electron microscopy
	S.G. Lawrence M.H. Holoka	test development - cadmium, zinc	algae, protozoa - population levels and cell weights - protozoan as a predator on algae
	S. Leonhard	test development	*Orconectes virilis* (crayfish) - mortality, moulting and toxicant uptake
		test development	*Artemia salina* (brine shrimps) - hatching and mortality

Location	Person or Group	Material	Parameter
		test development - chlorine, heavy metals, saline groundwater	*Daphnia magna* - reproduction and mortality
	W.R. Lillie S.E. Harrison W.A. MacDonald J.F. Klaverkamp	test development - acidification	*Brachydanio rerio* (zebrafish) - reproduction and mortality
	E.L. Lockhard A.P. Blouw	test development - herbicides	*Lemna minor* (macrophyte) - phytotoxicity - frond development
	W.L. Lockhart D.A. Metner	test development - pesticides, industrial chemicals, fish diseases	fish - biochemical and hemotological tests for "stress" cytochrome P-450, O - demethylase, δ-amino levulinic acid dehydrase, carbonic anhydrase, formation of microtubules
	W. MacDonald	test development - fenitrothion, copper, vanadium, zinc	rainbow trout, lake whitefish - static, flow-through embryo-larval tests - mortality
	M.A. Giles	test development - cadmium	rainbow trout - growth, biochemistry, histopathology, hematology, respiration, acid-base balance, osmoregulation
	J.F. Klaverkamp	test development - copper, vanadium, radionuclide metals	rainbow trout - kidney, liver, blood, gill enzymes - catalase, xanthene oxidase, carbonic anhydrase

Location	Person or Group	Material	Parameter
	J.F. Flannagan	test development - pesticides for blackflies	*Hexagenia rigida* (mayfly) - physiological functions - respiration
			aquatic systems - streams - production, standing crop, recolonization, emergence, drift
	D. Povoledo	Sulphur containing compounds, phosphoric acid herbicides	*Cyprinids* - thyroid - hypophysis hormone imbalance *Tetrahymena pyriformis* (protozoan) - and fish toxicity and uptake
	J.W.M. Rudd	test development - cadmium, mercury	microbes - chironomid - food-chain transmission and toxicity
	W.G. Franzin, M. Giles	cadmium, zinc	fish - polluted and experimental lakes - physiology, histology, biochemistry, growth, fecundity, population dynamics
	R. Wagemann W.G. Franzin	arsenic	fish - residues - Yellowknife, Northwest Territories
	J. Barica	copper	algae and fish of copper treated farm lakes - toxicity, uptake, copper cycling in lake
	J.W.M. Rudd	mercury, selenium	fish, invertebrates - interaction on mercury uptake
	H.E. Welch, J.W.M. Rudd	methane	arctic lakes - ecological impact

Location	Person or Group	Material	Parameters
	R. McV. Clarke	municipal wastes, chlorinated and non-chlorinated	fish - acute lethal and sub-lethal toxicity
		mine wastes	fish and macrobenthos - toxicity
	D.P. Scott	Statistical methods development	biometrics of acute bioassays
H ALBERTA			
I Fisheries and Environment Canada, EPS, Edmonton	B.T. Thackeray	drilling fluids and components	fish - marine - toxicity - on site testing
	B.T. Thackeray	effluent monitoring and surveillance	fish, invertebrates, algae
II Alberta Environment, Edmonton	W. Lake	effluent monitoring and surveillance	rainbow trout
		simulated fertilizer plant effluent	rainbow trout - toxicity
III University of Alberta, Edmonton Dept. of Civil Engineering	S. Hrudey	fatty acids	rainbow trout - toxicity
I BRITISH COLUMBIA			
I Fisheries and Environment Canada			
a) Pacific Environment Institute, North Vancouver	J. Davis, G. Greer	pulp and paper effluents	fish - chronic toxicity *in situ*
		copper	fish - chronic toxicity

Location	Person or Group	Material	Parameters
	H. Rodgers, J. Davis	land-fill leachates pulp mill effluents	fish - chronic toxicity, biochemistry, bioaccumulation
		halogenated compounds	fish, invertebrates - bioaccumulation
b) Fisheries Operations, Vancouver	I. Birtwell	pulp mill effluents	fish - avoidance, preference *in situ*
c) EPS North Vancouver	R. Watts	industrial effluent, and chemicals	fish - acute toxicity effluent monitoring
II International Pacific Salmon Fisheries Commission, Chilliwack (Cultus Lake)	J. Servizi, B. Martens	2,4-D	sockeye, pink and coho salmon, rainbow trout, acute and chronic toxicity
III Simon Fraser University, Burnaby, Dept. of Biology	B. Hardwick	oil and oil dispersants	invertebrates (bivalves) - *in situ* and lab chronic toxicity
IV University of Victoria Dept. of Biochemistry	A.T. Matheson	copper, zinc	coho salmon - biochemistry, chronic toxicity, interaction with infectious disease
	R.H. Mitchell	industrial hydrocarbons, pesticides	marine organisms - biochemistry, accumulation
	D. Ellis	trace metals (mines)	marine ecosystems - biochemistry, distribution, concentrations and effects
V EVS Consultants North Vancouver	G. Vigers	oil and oil dispersants	herring - acute toxicity, chronic effects, growth, feeding

Location		Person or Group	Material	Parameters
			pulp and paper effluents	herring and salmonids, acute and chronic effects - *in situ* and lab
			heavy metals in sediments	bivalves - chronic effects
VI	B.C. Reseach, UBC, Vancouver	T. Howard	pulp mill effluent constituents	fish - acute effects and biochemistry
VII	Beak Consultants, Vancouver		sulfite mill effluents	fish - waste treatment effects on acute toxicity and biochemistry
VIII	CEPEX, UBC	T.R. Parsons	organohalogens, copper, mercury and oil	microcosms - marine food chain - chronic effects

APPENDIX III

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FIFTH ANNUAL TOXICITY WORKSHOP

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